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A DECADE WITH THE COMMON COLD[1]

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[In the published version of this lecture it is proposed to summarise briefly, with references, those parts of the work on the Common Cold which have already been described in print and to write out fully the account of more recent work.]

The title of this lecture is not intended to refer to a description of a case of unusually intractable catarrh, but to describe ten years' work on the Common Cold at the Common Cold Research Unit at Salisbury in southern England. Actually, I am unduly modest, for we have been at it for over 11½ years.

The Common Cold is not amenable to study by simple laboratory techniques and so one has to detect the presence of the virus and to study it by the use of human volunteers inoculated intranasally. The primary objective has always been to discover some simple method of study which would eliminate the need for volunteers, at least for most of the work.

During these 11½ years more than 5,000 volunteers have passed through our hands. They are housed in pairs in comfortable one-story buildings and remain in isolation for 10 days; they are allowed to go out for walks but must avoid the other pairs and other human contacts. They have 4 days' quarantine, for preliminary observation and to make sure they are not already incubating a cold. They then receive intranasal installations of material under test—washings from other colds, 'control' innocuous fluids or the results of attempted virus cultivation. The volunteers do not know the nature of the inoculum, nor does the clinical observer until he has committed himself in writing as to whether a cold is present or not (1) (2). In our experience very few experimental colds are febrile; by far the most useful criterion has been the amount of nasal secretion; this is largely assessed by the numbers of paper handkerchiefs used daily. Numbers of colds produced by 'control' inocula have recently been as low as 1 per cent, whereas 'cold' washings induce the disease in as many as 50 per cent of inoculated people, the number varying somewhat from year to year.

Since washings from induced colds will again produce the disease whether they are filtered or not, we clearly have to deal with a multiplying, filterable agent, and this has the properties of a virus. It survives well at $-76°$ and has a diameter as determined by filtration, of 60 mμ or less. Attempts at cultivation in fertile eggs or at transmission to animals of many species have been unsuccessful (1).

[1] A De Lamar Lecture delivered at The Johns Hopkins University School of Hygiene and Public Health on February 21, 1958.

TRANSMISSION OF COLDS BY CONTACT

Experiments at transmission of colds from an infected to a normal person gave a low percentage of 'takes'. Attempts were made to get better results using 12 students isolated for 3 months on an island off the north coast of Scotland (4). This was done because of the many reports that arctic explorers and others in isolation for some months acquire greatly enhanced susceptibility to colds. Alas, when we brought 10 people with early induced colds on to the island at the end of the 3 months and exposed them under various conditions to our volunteers, no transmission occurred. We thought it possible that our 'pedigree' serially transmitted colds might have lost the power of being transferred by natural contact; this idea was confirmed when a crofter with a naturally acquired or 'wild' cold, did transmit his colds to three of our students. We followed this observation up in more experiments at Salisbury. Epidemiological evidence had suggested that colds are apt to spread more readily from children than from adults (12). So children with acute natural colds were invited from the neighbouring Chalke valley to special children's parties, where they had the opportunity to spread infection in various ways to adult volunteers. Close contact permitted cross-infection to about 10 per cent of exposed persons; transmission by means of minute 'droplet nuclei' was nearly as effective; exposure to a contaminated environment gave equivocal results (13).

Other experiments indicated that the cold virus is readily inactivated by drying, that use of dried infected handkerchiefs does not readily convey infection and that application of infected secretions to the outside of the nostrils is also relatively ineffective.

We failed to produce colds by chilling and similar methods, nor by such means to increase susceptibility to a small dose of virus (2). Possibly the prevalent belief that chilling causes colds may confuse cause and effect; a feeling of chill on exposure to, say, a draught may be a first symptom of a cold and not its cause. Dowling *et al.* (10) have lately reported that chilling can precipitate a cold but only in women at a particular phase in the menstrual cycle.

TISSUE CULTURE

When we had wholly failed to infect any animal or to cultivate a virus in eggs, we decided, in 1952, to concentrate on attempts to grow the cold virus in cultures of human tissues. We used tissues from embryos obtained from therapeutic abortions; also adult nasal tissues obtained at biopsies. Some earlier work had given a little promise, and by 1952 the possibilities of tissue culture for growing 'difficult' or 'new' viruses were just beginning to appear. For a year we had no success. Then in 1953 we obtained evidence that one line of cold virus (DC) could be propagated in cultures of human embryonic lung (3). It

was carried in series for ten passages, producing colds over a period of three months in 13 of 92 volunteers (14 per cent), the later passes doing as well as the earlier. No cytopathic effects were detected in the cultures, but we felt it to be only a matter of time before other evidence of virus growth could be detected. The results were reported at the International Microbiological Congress in Rome in September, 1953, and that finished it! Thereafter we had only infrequent 'takes' with culture material and only in very early passages. We do not know at all why the DC virus apparently grew for a while and was then lost. We varied every factor we could think of. It is perhaps relevant, however, that during the period of success we were making passages in cultures of one particular—but in no way unusual—embryo. When that was used up, we had only failures.

IS THERE 'A' COMMON COLD VIRUS?

Quite recently there have been discovered other viruses having probable relationships to minor upper respiratory infection, all of them propagable in tissue culture. Before considering these, let us enquire whether there necessarily exists a single common cold virus or whether the causes of colds may not be many and various. I have referred to the properties of 'the' cold virus, but I recognize that the results may be deceptive if colds are caused by several unrelated agents. There is already evidence of diversity of properties. We have encountered a strain of colds which has a longer incubation period than usual, about 4 instead of 2 or 3 days; this breeds true on serial passage in volunteers (7). From earlier experiments we concluded that 'the' cold virus was readily inactivated by 20 per cent ether: recent tests with another strain have suggested that it is ether-resistant. On the other hand there are experiments (9) which indicate that immunity to a single strain of colds is of very brief duration, so that there would seem to be no evolutionary reason for development of antigenic diversity. If causes of colds were multiple, several anomalies would disappear. Thus there are numerous reports that cold epidemics promptly break forth amongst Arctic explorers who, after long isolation, unpack bales of clothing. Such records suggest that some cold viruses persist very well in the dried state, a result in complete contrast to our own experimental findings with the strains we studied.

VARIOUS RESPIRATORY VIRUSES

The adenoviruses, discovered by Rowe *et al.* (19) and by Hilleman and Werner (11) are associated with pharyngitis and febrile catarrhs, infections clinically severer than common colds: serological types 3, 4 and 7 are especially to blame. We tested at Salisbury cultures of type 1, at that time reputed to be harmless to adults. Several infections were produced, definitely more severe than our experimental colds (18). There resulted also some typical colds which on passage

bred true as such, though of the variety with a longer incubation. Washings from these failed to yield adenoviruses. Presumably we had either activated a latent cold-infection by introducing our adenovirus, or had picked up such a virus from a volunteer, this having, because of its longer incubation, slipped past our regular 4-day preliminary observation period. Activation of one virus by another remains an intriguing possibility: it is an occurrence familiar to workers with viruses attacking insects.

Other viruses deserve brief mention. There are two, reported respectively by Sabin (20) and by Morris *et al.* (15) associated with colds in chimpanzees. With each of them there are suggestions of pathogenicity for man. Morris' virus, further studied by Chanock (8) and called CCA (Chimpanzee coryza agent), has been isolated from infections in children; antibodies against it are present in many human sera. It produces cytopathic effects in monkey kidney tissue cultures. Sabin's virus is related to a virus which was at first classified, perhaps wrongly, as an echo virus, is ether resistant and present in the stools (20).

Chanock *et al.* (7) have isolated other agents which they call HA (haemadsorption) viruses, since cells in tissue cultures infected with them adsorb certain red blood cells. They seem to be myxoviruses (i.e. related to the influenza-mumps group). Similar viruses may be present in cultures of 'normal' monkey kidneys—which seem at the moment a limitless source of 'new' viruses. Two other viruses, one sent to us from Scandinavia, another isolated by Tyrrell and Worthington at Salisbury, are under study as agents which can be cultivated in monkey kidney but whose relationship to colds is still quite uncertain. The CA (croup-associated) virus is another 'new' virus of the Myxovirus group (6), (5).

JH AND 2060 VIRUSES

These are two viruses, isolated independently by Winston Price (16) and Mogabgab and Pelon (16), cultivable and causing cytopathic effects in monkey kidney cultures but growing with some difficulty, preferring a medium without serum and not yet shown to cause haemagglutination. Though otherwise similar, the two strains are serologically different (personal communication from Dr. Winston Price). The JH virus seems to be associated with minor infections in children; yet these infections were febrile in 23 of 26 children and not typical of colds (17). Prophylactic vaccination against the infection was reported to be effective. In view of suggestions that the JH virus was related to colds, Drs. Tyrrell and Bynoe (paper in preparation) carried out experiments on our volunteers at Salisbury with a virus kindly sent us by Dr. Price. The results are shown in the figure. In two experiments, and in a third not recorded, cultures of 9th monkey kidney passage, given at the dilutions shown, produced no colds in any of 16 volunteers. Virus was, however, recovered from nasal washings of two of them and one of these washings produced two colds, which again

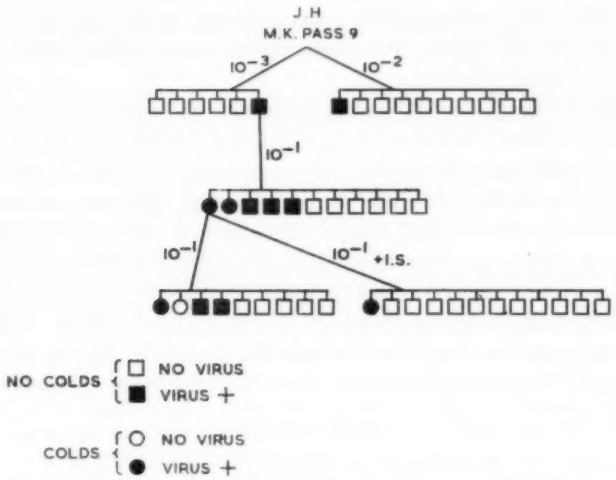

FIG. 1. Results of inoculation of human volunteers with JH virus.

yielded virus. Three other volunteers, who developed no colds, also had virus in their washings. A further passage yielded a similar result except that on this next test one volunteer developed a cold yet no virus was recovered. This failure may be ascribed to insufficient sensitivity of the test or to our having picked up another cold virus, as perhaps happened in the experiment with adenovirus mentioned earlier. The figure also shows that a high titre anti-JH rabbit serum failed to neutralize a positive washing completely. The results suggest that JH virus has lost pathogenicity for man after cultivation in monkey kidney but that it may be re-activated by intranasal passage of washings in man and produce colds. This conclusion must be tentative as the results in the figure will hardly bear statistical analysis. Evidence that JH virus causes colds in adults is therefore equivocal.

Two interesting points arise. If the JH colds are genuine, it seems possible that a virus causing febrile illnesses in children may produce common colds in adults, who presumably have some immunity. Possibly several viruses can act thus, for the febrile catarrhs of children are many and various. Epidemiological studies near Salisbury, undertaken by Dr. A. T. Roden (personal communication), do however suggest that some colds in adults produce in small children to whom they may spread, the typical picture of an afebrile running cold.

The other point is this: some viruses are known to multiply in tissue cultures without leading to evident cytopathic effects. It now appears possible that a virus may rapidly lose pathogenicity for man in culture—as indeed influenza virus does on cultivation in eggs. It is therefore possible that the difficulty in cultivating common cold viruses is that this virus may suffer from

both troubles, losing pathogenicity on culture and at the same time failing to damage the cultures visibly. We might therefore be growing cold viruses all the time and never know it!

To conclude: new methods are revealing new respiratory viruses one after another. The outlook for increasing our knowledge is hopeful. We do not know yet whether colds will prove to be due to numerous viruses of perhaps equal importance or whether these 'new' viruses are on the periphery of the problem, there remaining a hard central core of 'the' common cold virus. It may also be that with cold viruses as with influenza A and perhaps adeno- and echo-viruses, there is a constant turnover, one after another coming to dominate the scene.

The results from Salisbury have been the result of team work. Workers concerned during the last few years include M. L. Bynoe, D. M. Chaproniere, J. Field, H. G. Pereira, A. T. Roden, D. A. J. Tyrrell and G. Worthington.

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BONE MARROW AND FAT EMBOLISM IN SICKLE CELL ANEMIA AND SICKLE CELL-HEMOGLOBIN C DISEASE

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INTRODUCTION

The purpose of this report is to call attention to the occurrence of infarction of the bone marrow resulting in massive embolization by bone marrow and fat in sickle cell anemia and sickle cell-hemoglobin C disease. Recently three cases illustrating this course of events have come to autopsy at the Johns Hopkins Hospital. The medical literature contains reports of four, or possibly five, isolated instances of fat embolism associated with sickle cell diseases but in none of these was the genetic hemoglobin pattern known; in none was the diagnosis of a sickle cell disease established before post-mortem examination; and in none was actual bone marrow embolism demonstrated.

Terminology: The term "sickle cell disease" is used broadly to include the entire spectrum of disorders whose manifestations are related to the presence in the erythrocyte of type S hemoglobin.

With regard to the cases recorded in the literature and reviewed in this report, the finding of "sickled red blood cells" in the tissue sections is accepted as evidence indicative of the presence of type S hemoglobin.

The term "sickle cell anemia" is used specifically for the disorder characterized by the homozygous state for the hemoglobin S gene. The hemoglobin electrophoretic pattern for this state is represented by the symbol S-S. Sickle cell-hemoglobin C disease represents the disorder characterized by the heterozygous state for type S and type C hemoglobin. The hemoglobin electrophoretic pattern for this state is represented by the symbol S-C.

REPORT OF CASES

Case No. 1 (JHH #379041). A 41 year old negress registered in the obstetrical clinic of The Johns Hopkins Hospital in the 24th week of her third pregnancy on June 5, 1957. Both previous pregnancies had ended in spontaneous abortion during the first trimester. She stated that a sister and a cousin had "sickle cell anemia." The past history disclosed an untreated luetic infection in 1954 and "german measles" in about the 15th week of the current pregnancy. She had been seen in the accident department of this hospital on several occasions during the preceding several years complaining of rather vague aches and pains in the muscles, bones, and abdomen. The cause of these symptoms was not determined. The initial prenatal physical examination was normal. The hematocrit was 29.5 per cent. The

[1] On leave from the Department of Gynecology.

serological test for syphilis was positive. Treatment with penicillin was instituted and completed without event. In the 35th week she began to complain of joint pain which soon involved both forearms and legs. A hematocrit value of 27.5 per cent was found, and blood for hemoglobin electrophoresis was drawn. On September 8, 1957 she came to the accident room, two weeks from term, complaining of severe pain in the back. She was admitted to the delivery floor. Vital signs included a temperature of 99.8°, pulse 92, and blood pressure of 125/60. She continued to complain of severe back pain and seemed quite uncomfortable. No signs of labor were present. No other abnormal findings on physical examination were noted. The hematocrit on admission was 23.5 per cent. Low back, arm and leg pain continued but she seemed somewhat improved. Twenty hours after admission the temperature rose to 100.6° and rales and rhonchi were noted over the right lower lung field. Twenty-four hours after admission, one million units of penicillin were given intramuscularly at a time when the temperature was 102° and respirations were 24 per minute. At this time the report of the hemoglobin electrophoresis established the diagnosis of sickle cell-hemoglobin C disease. Two hours after the administration of the penicillin, shortness of breath, tachycardia, sweating and slight elevation of the blood pressure were noted. These symptoms progressed rapidly and musical wheezes became generalized over the chest. There was no skin rash or urticaria. The ocular fundi were normal. The hematocrit was found to be 27 per cent, the WBC 22,000 and the serum bilirubin was 2.9 mgms. per cent, 1.8 of which was directly reacting. In spite of adrenalin, aminophylline, cortisone and digitalization, deterioration continued. Moist basilar rales were heard. One hour before death she became disoriented. Thirty minutes later the fetal heart sounds disappeared. The blood pressure and pulse disappeared and shortly afterwards respirations and cardiac action ceased.

After the death of the patient, but prior to autopsy, the diagnosis of widespread infarction of the bone marrow with subsequent embolization of fat and bone marrow was suggested because of experience with two similar cases, both of which are included in this report.

A complete post-mortem examination (Autopsy #27553) was performed eight hours after death. The body was that of a normally proportioned negro female of average height with a term intrauterine pregnancy. There was no scleral icterus. The heart was normal. The lungs weighed 1120 grams and felt slightly heavy. There were no remarkable gross changes externally or on the cut surfaces. The pulmonary arterial tree contained what was thought grossly to be post-mortem clot. The liver weighed 2150 grams but was otherwise not remarkable. The spleen weighed 1400 grams. The capsule was thickened. The cut surface was a bright, homogeneous maroon color. There were no visible areas of scarring. The kidneys were normal. The ureters were three times normal nulliparous diameter. A 3100 gram, well developed female infant was present in a term uterus with intact membranes. Electrophoresis of the fetal blood revealed a combination of normal and fetal hemoglobins. The vertebral, rib, and sternal marrows were irregularly red in color with what seemed to be hemorrhagic areas. Necrosis was not grossly appreciated. The femoral marrow was red and soft.

Microscopic study: The spleen showed engorgement with red cells compressing sinusoids and lakes of red cells surrounding small follicles. Focal extramedullary hematopoiesis was present. No scarring or hemosiderin deposition was identified. No hemosiderin was present in the renal tubular epithelium. The glomerular capillaries were engorged with red cells.

The marrow of the vertebra, sternum and rib contained numerous areas of recent infarction and hemorrhage. The small intramedullary vascular channels were engorged with tangles of sickled erythrocytes. No distinct thrombi were seen. Most interesting were large veins just outside the cortex of the sternum which were plugged with masses of necrotic bone marrow identical with that seen in the intramedullary cavity. The ribs and femoral marrow contained smaller areas of fresh necrosis.

Necrotic bone marrow including several bony spicules were present in many of the large, medium, and small pulmonary arteries. Sections of what had been thought at autopsy to be post-mortem clot in the pulmonary arteries showed the presence of necrotic marrow.[2] Fat could be seen in the necrotic marrow and filling many of the pulmonary arterioles and capillaries. There was slight pulmonary edema in some of the sections. Nearly every renal glomerulus contained fat globules in the glomerular tuft. Sections of the brain showed the presence of a considerable number of intravascular fat globules.

Anatomical Diagnosis: History of sickle cell-hemoglobin C disease. Normal term fetus in utero. Pregnancy changes in the breasts, pituitary and ovaries. Sickle cells in placental sinusoids. Splenomegaly with hemorrhagic pulp compressing sinusoids. Extramedullary hematopoiesis, spleen and liver. Hyperplasia, femoral marrow. Focal hemorrhages containing sickle cells, bone marrow. Focal infarct-like necroses, bone marrow. Bone marrow emboli, parasternal veins. Massive embolization of necrotic bone marrow, bone trabecula and fat, lungs. Fat emboli, renal glomeruli and brain. Pulmonary edema. Pleural effusions. Myomata uteri. Focal hemorrhages, ureters. Calcified mediastinal lymph node.

Case #2 (JHH #328902). A 15 year old colored male was admitted to the Plastic Surgical Service of The Johns Hopkins Hospital for the third time on June 6, 1956 for plastic revision of scar contracture of the left ankle resultant from skin ulcers which had been previously successfully grafted.

In 1948 at the age of seven years he was seen in the Out-Patient Department of the Harriet Lane Home for pain in the right hip and thigh. A hemoglobin determination was 8.5 grams and a sickling prepration showed complete sickling in five hours. He was observed by this clinic until the age of 12 years. Anemia and slight jaundice were constant features of his disease. Sore throats were frequent and on several occasions were accompanied by mild crises manifest by pain in the extremities, arthralgia and arthritis. In 1953, age 12 years, ulcers over the ankles appeared. Successful skin grafting was performed. From this time until his death, he was followed in the Hematology Clinic. Slight cardiomegaly with a soft diffuse systolic murmur were noted. The hematocrit ranged from 23 per cent to 27 per cent, the icterus index from 25 to 75, the serum bilirubin from 4.7 to 5.5 mgm. per cent and the reticulocyte count from 6.6 to 9.6. Hemoglobin electrophoresis showed a S-S pattern. He did generally well and complaints of pain in the extremities and mild epigastric discomfort were infrequent. Regrafting of the leg ulcers was necessary once in 1955.

On admission the hematocrit was 22 per cent and the WBC was 9,800. On June 7, 1956 several "Z" plasties under local anesthesia to break the contractures were carried out. One 500 cc. transfusion of whole blood was given in the immediate postoperative period and the hematocrit rose to 26. His course was uneventful until June 12 when he complained of arthralgia and the temperature was 102°. Another 500 cc. transfusion was given. On June 13 he continued to complain of multiple aches and pains in the joints and extremities and was noted to be quite lethargic. The temperature was 103°. A hematocrit determination was 26 per cent, the WBC was 22,000 and the serum bilirubin was 8.5 mgm. per cent, 4.2 of which was directly reacting. Symptomatic measures were instituted. Lethargy deepened. Arthralgia and malaise continued. A tachycardia of 120–130 without evidence of heart failure was noted for 10 hours before death. He died rather suddenly in the early morning of June 14, 1956. The clinical diagnosis was terminal sickle cell crisis.

A complete autopsy was performed 6 hours after death. (Autopsy #26535). The body was that of a well developed and well nourished, muscular 15 year old negro male. The recent

[2] Fat was identified in the various locations described by the use of frozen sections and staining with either Sudan III or Oil red O.

surgical incisions about the left ankle were clean and healing. The sclerae were icteric. The ventricular chambers of the heart were slightly dilated. The lungs were light and grossly normal. The liver was enlarged, weighing 2025 grams. It was engorged with blood and fine gross scarring was apparent. The spleen was small, weighing 60 grams; otherwise, it was grossly normal.

Microscopic study: There was impressive engorgement of small blood vessels in the organs. Numerous sickled erythrocytes were present. The liver showed changes of early "sickle cell cirrhosis" (1). The spleen was congested with lakes of blood in and about the Malpighian bodies. No scarring or hemosiderin deposition was present. The renal tubular epithelium contained considerable hemosiderin.

Sections of the vertebra, rib and sternum all showed, in addition to marked sinusoidal engorgement by tangles of sickle cells and hyperplasia, numerous foci of fresh necrosis. This was more extensive in the section of sternum where the necrotic marrow could be clearly seen passing into large venous sinusoids running through the cortex of the bone and into surrounding veins. All sections of the lungs showed numerous small fragments of both necrotic and well preserved bone marrow in the small pulmonary arteries. There was widespread fat embolization of the pulmonary capillaries. Fat was also present to a striking degree in the renal glomerular capillaries and to a less impressive but demonstrable degree in the capillaries of the brain.

Anatomical Diagnosis: Sickle cell anemia. Sickle cell spleen and cirrhosis. Hemosiderin deposition, renal tubular epithelium. Hyperplasia, bone marrow, with focal infarctions. Widespread fat and bone marrow embolization, lungs. Fat emboli, kidneys and brain. Dilatation and hypertrophy, right and left ventricles. Ulcers, both legs. Hyperplasia, thymus.

Case #3 (JHH #506167). A 14 year old negro male was admitted to the Medical Service of The Johns Hopkins Hospital for the first time on April 20, 1954 because of fever, chills, right pleuritic chest pain and a cough productive of greenish sputum of two days duration.

He had been known to have sickle cell anemia since early childhood and recent hemoglobin electrophoresis had revealed an S-S pattern. There had been numerous previous admissions to the Harriet Lane Home for hemolytic crises accompanied by joint and abdominal pain, epistaxes and repeated respiratory infections, including episodes of sinusitis and pneumonia. Over the years the hemoglobin ranged between 5 and 9 grams. There had been slight jaundice and reticulocytosis. At the age of eight years, acute rheumatic fever was diagnosed and since then there had been a persistent harsh systolic murmur and moderate cardiomegaly. The last known transfusion had been received three and one-half months prior to admission.

On admission the temperature was 101°, pulse 84, respirations 20 and the blood pressure 96/84. He was underdeveloped with disproportionately long extremities and in no acute distress. The heart was moderately enlarged and there was a harsh systolic murmur heard all over the precordium. The second pulmonic sound was louder than the second aortic sound. Coarse rales were heard at the right lung base posteriorly. A chest x-ray showed a right middle lobe infiltrate. The hematocrit was 20 per cent and the WBC was 20,000. The icterus index was 10 and the bilirubin was 1.4 mgms. per cent with less than 0.8 being directly reacting. A heavy growth of gamma streptococci was cultured from the sputum.

Five hundred cc. of whole blood were given on the day of admission. Achromycin, 2 grams per day, was started. Daily temperature elevations to 101°–102° continued. A chest x-ray on April 28 showed clearing of the right middle lobe infiltrate but the temperature elevations continued. Cobaltous chloride was given a seven day trial but was discontinued because of tachycardia. On May 5, a series of epistaxes began. Because of these, the persistent fever on antibiotics in the face of clearing the chest x-ray, and the past history, the diagnosis of active rheumatic fever was suggested. Aspirin therapy was initiated and the temperature

fell to normal levels in the next few days. An antistreptolysin O titer was reported as 2,000 Todd units. He was asymptomatic by May 20 and remained so until June 1. A circumcision was carried out on May 20 without event. On June 1 several teeth were extracted. Shortly after his return from the dental clinic, he began to complain of moderately severe right upper quadrant pain which was non-radiating and constant. The temperature was 101.4°. Respirations were 34 per minute. There was moderate right upper quadrant and CVA tenderness. The hematocrit was found to be 21 per cent and the icterus index 50. Two days previously the hematocrit was recorded as 27 per cent and the icterus index as less than 5. Shortly afterwards, he complained of pains in the shoulders, arms and knees and became lethargic. The sclerae were icteric. The temperature remained elevated. On June 3, the hematocrit was 23 per cent. The total bilirubin was 28.8 mgm. per cent of which 20 mgm. per cent was directly reacting. Rapid respirations and lethargy continued and he became restless and combative. No signs of heart failure were present. On June 4 the hematocrit was 17 per cent and the bilirubin was 40 mgm. per cent with a direct bilirubin of 28.8 mgm. per cent. Shortly before death on June 4, he became unresponsive and the temperature rose to 105°. Death was attributed to sickle cell crisis.

A complete autopsy (Autopsy #25089) was performed three hours after death. Deep jaundice was present. The heart was enlarged due to hypertrophy of the left ventricle and dilatation of all chambers. The mitral valve was thickened and its chordae tendineae were thickened and shortened. There was a small hemorrhagic infarct in the upper lobe of the right lung. Otherwise the lungs showed no gross lesions. The liver was enlarged, swollen and relatively soft. The spleen was small and scarred, weighing 34 grams. Gross lesions were not appreciated in the bone marrow.

Microscopic study: There was widespread vascular engorgement with sickled erythrocytes. The spleen showed characteristic scarring and hemosiderin deposition. Hemosiderin was deposited in the renal tubular epithelium. In the liver, in addition to marked sinusoidal congestion, the Kupffer cells were stuffed with phagocytized erythrocytes and there were widespread focal necroses of hepatic cells. There was a prominent periportal round cell infiltration. Aschoff bodies were present in the endocardium of the left atrium and in the myocardium.

In addition to vascular engorgement in the bone marrow, focal necroses were present. There were numerous necrotic bone marrow emboli in the medium sized and small pulmonary arteries. There was a small hemorrhagic infarct in the right upper lobe. Fat embolization of alveolar capillaries was marked. There were numerous fat globules in the renal glomerular capillaries. Intravascular fat was not demonstrated in the brain.

The fall in the hematocrit and the changes in the tissues indicate that the patient was in hemolytic crisis during the final events. However, the degree and nature of his jaundice were due in part to the changes in the liver. The necroses can be attributed to severe impairment of hepatic blood flow by the combined effects of anemia and sinusoidal obstruction by sickled red cells and Kupffer cells engorged with phagocytized red cells (1). Although these processes seem to be the most likely explanation for the liver cell necroses in this case, the finding of a marked periportal round cell infiltrate has not been a feature of the liver changes in the sickle cell diseases, even in cases that have progressed to marked cirrhosis. For this reason, the possibility that there may be superimposed epidemic hepatitis or homologous serum jaundice resultant from prior transfusions cannot be completely excluded. In spite of the marked changes in the liver, the bone marrow and fat emboli were probably major contributory factors in causing the death of this patient.

Anatomical Diagnosis: History of sickle cell anemia. Hemorrhages, scarring and Gamma-Gandy body formation, spleen. Marked sinusoidal congestion and phagocytosis of sickle

cells by Kupffer cells. Widespread focal necroses, liver cells. Periportal mononuclear infiltrate, liver, (etiology ?). Jaundice. Pigmented casts, renal tubules. Hemosiderin, renal tubular epithelium. Marked vascular engorgement, vessels of marrow with focal marrow necroses. Bone marrow emboli, lungs. Small pulmonary infarct. Fat emboli, lungs and renal glomeruli. Rheumatic heart disease. Hyaline vegetation and scarring of mitral valve. Scarring and Aschoff bodies, left atrium and Aschoff bodies, myocardium.

CASES FROM THE LITERATURE

Possibly the earliest report associating fat embolization and a sickle cell disease is that of Brittingham and Phinizy (2) in 1931. This case was reported as one of "Hemorrhagic Encephalitis" after neoarsphenamine therapy.

The patient was a 20 year old negress who was being treated with neoarsphenamine for latent syphilis. Shortly after an injection of neoarsphenamine she complained of sudden and severe back pain. This lasted only a short while, but two days later she was admitted to the hospital in coma and died shortly thereafter. The autopsy disclosed multiple small hemorrhages in the central nervous system. The spleen weighed 230 grams. The red cells were sickled in the sections and there was widespread fat embolization. No source for the fat emboli was found.

The first well documented case appearing in the literature in which bone marrow necrosis and fat embolization was observed with a sickling abnormality was reported in 1941 by Wade and Stevenson (3).

A 49 year old white Greek female suddenly experienced severe back pain which was followed by vomiting. The pain returned in paroxysms over the next three days and on the fourth day following the onset of the pain, there was a shaking chill followed by coma. The past history was of interest in that six years previously she had been surgically explored with the preoperative diagnosis of gall bladder disease when she presented to the hospital with right upper quadrant pain and tenderness, anemia and slight icterus. The exploration was negative but the symptoms subsided. "Sickled" red cells were noted in the blood smear and a large number of nucleated red blood cells were also seen. Since that time there had been several episodes of "pain" and paresthesias sometimes associated with "darkening" of the skin. The patient on admission was critically ill and semi-comatose. Petechiae were noted on the skin and conjunctivae. The hemoglobin was 40 per cent. There was anisocytosis, poikilocytosis and a large number of nucleated red cells were present in the blood smear. The icterus index was 15. Her condition was essentially unchanged for 10 days until she became more deeply comatose and died. The autopsy showed widespread necroses in the bone marrow. There were fat emboli in the lungs, kidneys and brain. Necroses were present in the brain. The spleen weighed 350 grams, and showed scarring with iron incrustations. Sickled red cells were noted in the sinusoids.

Later in 1941 Vance and Fisher (4) reported a similar case.

A 49 year old negro female who had previously enjoyed good health experienced sudden, severe low back pain. Twenty-four hours later she became stuporous and was admitted to the hospital in coma. Vital signs included a temperature of 102°, respirations 26, pulse 98 and blood pressure of 120/80. She remained in coma and died twelve hours later. Necropsy revealed a small, 30 gram spleen in which there was scarring and iron incrustations. Sickled erythrocytes were noted in smears prepared from the spleen. There were necroses in the bone marrow and fat emboli in the lungs, brain and kidneys.

In 1942 Wertham (5) reported the following case.

A 28 year old negro female who was described as being "mentally peculiar" was admitted to the hospital because of the onset of stupor. She had complained of intermittent migratory polyarthritis for 5 years. Five days prior to admission she developed an upper respiratory infection with cough, chills and fever. On the day of admission, she developed pain "all over the body" especially in the arms, legs and shoulders. She became stuporous and was admitted to the hospital. Scattered petechiae in the skin, conjunctivae and eyegrounds were noted. She died on the ninth day of her illness after developing a left hemiparesis and convulsions. The autopsy showed a large spleen which histologically was congested with sickled erythrocytes and contained occasional iron incrustations. Sections of the bone marrow showed no necroses and fat was present in the lungs, kidneys and brain.

The most recent case appearing in the literature is that of Wyatt and Orrahood reported in 1952 (6).

A 27 year old pregnant negress entered the hospital two weeks prior to term because of severe pain in the arms, shoulders, and back followed by lethargy of three hours duration. Physical examination was not remarkable except for the lethargy. Dyspnea and slight icterus developed. Serum bilirubin was 2.4 mgms. per cent, most of which was indirectly reacting. Three days later a precipitous spontaneous delivery was followed by slight hemorrhage and increasing dyspnea and joint pains. Death followed in several hours. At autopsy, the spleen weighed 1220 grams. There were no other gross abnormalities. Microscopically, the spleen revealed intense congestion with sickled shaped erythrocytes. The hyperplastic bone marrow showed vascular engorgement and focal necrosis. Fat was demonstrated in the capillaries of the lungs, kidneys and brain.

SUMMARY OF THE CLINICAL FEATURES OF BONE MARROW INFARCTION AND BONE MARROW AND FAT EMBOLIZATION IN THE SICKLE CELL DISEASES

The clinical features of fat embolism following trauma to the fat deposits either in bone or soft tissue have been described by numerous authors (7). They include in addition to the history of trauma, mild tachycardia and tachypnea, shock, fever, mental depression, petechial hemorrhages, funduscopic changes

TABLE I

Summary of the Clinical and Pathological Features of Bone Marrow Infarction and Bone Marrow and Fat Embolization

Case	Age	Race	Sex	Ante-mortem diagnosis of a sickle cell disease	Hemoglobin electrophoresis	Associated with pregnancy	Bone pain	Lethargy	Fever	Petechiae	Spleen weight (grams)	Bone marrow necroses	Fat emboli	Bone marrow emboli
1. JHH-379041 Autopsy-27553	41	N	F	+	S-C	+	+	0	+	0	1400	+	+	+
2. JHH-328902 Autopsy-26535	15	N	M	+	S-S	0	+	+	+	0	60	+	+	+
3. JHH-506167 Autopsy-25089	14	N	M	+	S-S	0	+	+	+	0	34	+	+	+
4. Wade and Stevenson (3)	49	W (Greek)	F	0	—	0	+	+	+	+	350	+	+	0
5. Vance and Fisher (4)	49	N	F	0	—	0	+	+	+	0	30	+	+	0
6. Wertham et al (5)	28	N	F	0	—	0	+	+	+	+	"large"	0	+	0
7. Wyatt and Orrahood (6)	27	N	F	0	—	+	+	+	?	0	1220	+	+	0
8. Brittingham and Phinizy (2)	20	N	F	0	—	0	+	+	+	+	230	0	+	0

(8, 9), fat droplets appearing in the sputum and urine and the demonstration of large fat droplets in circulating blood (10).

Table I includes the outstanding clinical features of bone marrow infarction followed by embolization of bone marrow and fat associated with one of the sickle cell diseases observed in our three cases and in those gathered from the literature. In this group, bone pain was the outstanding initial complaint and was present in all cases. Varying degrees of mental depression and fever were almost constant features. Petechiae, which would seem to be a valuable diagnostic sign, were not noted in our cases although they were present in the majority of the previously reported cases. It is of interest and perhaps of significance that two of the six females were pregnant and at or near term at the time of death. Recently three cases of rather sudden death occurring in pregnant women with sickle cell-hemoglobin C disease were reported from Ghana (11). No adequate anatomic explanation for death was described in the report.

In the experience of most (12), fat droplets are observed in the sputum and urine only 2–4 days after embolization occurs. Probably the most helpful and significant diagnostic procedure is the demonstration of large fat droplets (7–12 microns) by a procedure using a water soluble fluorescent fat stain, phosphin 3R, as outlined by Peltier (10) in his excellent report.

DISCUSSION

It is not intended to discuss the pathology of sickle cell anemia (S-S) or sickle cell-hemoglobin C disease (S-C) demonstrated in these cases other than

that which pertains to infarction of bone marrow and the subsequent embolization of bone marrow and fat. Since the advent of hemoglobin electrophoresis and the division of the sickle cell diseases into distinct genetic types, interesting clinical data have resulted. (13, 14) The clinical similarities and differences between the various genetic types are becoming more clearly defined. There is considerable overlap in the pathology within this group of diseases; however, on the basis of the changes in the tissues alone, one can frequently predict whether the case is one of sickle cell anemia or one of the genetic variants. Of the previously reported cases reviewed here, one can, on the basis of the age of onset of symptoms, clinical course, the hematologic data and the size of the spleen, reasonably deduce that all of them represent one or the other of the genetic variants of sickle cell anemia. It seems likely that the case of the 49 year old white Greek female reported by Wade and Stevenson represents sickle cell-thalassemia disease. The others are likely to be cases of sickle cell-hemoglobin C disease.

It is probable in the light of present knowledge that the underlying mechanism responsible for many of the clinical manifestations of a crisis in the sickle cell disease is the obstruction of vascular channels by tangles of sickled erythrocytes. The distention of the intramedullary cavity of the bones by this vascular engorgement likely accounts for the bone pain of a crisis. In some cases there may be focal infarction of the marrow. Such infarcted foci heal by scarring in which there is osteoid deposition resulting in the irregular sclerosis sometimes seen in the x-ray films of the long bones (15).

Necrosis of bone marrow is demonstrated in Figure 1. Infarcted and dislodged bone marrow fragments may then gain entry into venous channels within bone and pass out into the venous circulation. This is seen in Figure 2. The probable explanation for the passage of this material into the venous system has been reviewed by Rappaport in reference to bone marrow emboli found in cases of traumatic injury (16). Bone marrow is separated by only a single endothelial layer from intramedullary sinusoids. These sinusoids communicate freely and widely with veins which pass through cortical bone. They are large enough to permit passage of large fragments of such material. The veins do not collapse in the presence of surrounding injury since they are frequently firmly adherent in many places to bone. In such circumstances, the intramedullary pressure is greatly increased and augments expulsion of disrupted material into available receptors. Embolism of necrotic bone marrow and fat follows. Marrow fragments are arrested in the pulmonary arterial tree (Fig. 3). A small bony spicule found in the necrotic marrow embolus in the main pulmonary artery of Case No. 1 is illustrated in Figure 4. Fat globules may become arrested in the lungs. (Figs. 5 and 6). However, they may pass through the pulmonary capillary bed and into the peripheral circulation. Although intravascular fat may be demonstrated in any organ, it is most easily seen in the renal glomeruli. (Figs. 7 and 8). Of greater clinical significance, however, is fat embolism to the brain.

The fat emboli are not easily seen in the brain in routine paraffin sections and frozen sections stained for fat are necessary to conclusively demonstrate the emboli. In all the cases reviewed, signs of mental depression attributable to fat embolism were present except in Case No. 1 in which the magnitude of pulmonary vascular obstruction was sufficient to cause death before cerebral signs would be expected.

CONCLUSIONS

In view of the high incidence of the sickle cell diseases and the frequent occurrence of bone pain during crises, it seems likely that the apparent low incidence of marrow and fat embolism resulting from infarction of the bone marrow may be misleading. A review of the autopsy files of this hospital for a period covering the last thirty years failed to disclose another case in which fat or bone marrow embolism could be identified in the available material. This may be due to several factors. The diagnosis may be missed at autopsy since there are no striking gross changes and enough sections may not be obtained. For example, the material filling the main pulmonary arteries in Case No. 1 was thought by numerous observers at the autopsy table to be post-mortem clot; however, due to the awareness of the problem created by the two earlier cases, sections were taken of this material instead of discarding it as would be the usual procedure. A relatively slight degree of fat embolism may be easily overlooked in routine paraffin sections and therefore fail to stimulate staining for fat. And finally, non-fatal events occurring at remote times before death may leave no residue to be specifically diagnosed at autopsy. Authors (7, 12) writing on the subject of fat embolism associated with bone trauma stress the fact that many cases of fat embolism are overlooked clinically unless there is a particular awareness on the part of the physician of this complication and the resultant symptomatology. Although it is clearly established that many cerebral and pulmonary manifestations of the sickle cell diseases result from vascular occlusions due to tangles of sickled red cells (17, 18) it is suggested that examination of the blood for fat droplets during and after crises may show that fat and possibly bone marrow embolism account for these manifestations in some cases.

SUMMARY

Three cases in which bone marrow infarction was followed by embolization of bone marrow and fat are reported. Two of the three were of sickle cell anemia (S-S) and the third was of sickle cell-hemoglobin C disease (S-C). It was clearly the cause of death in two cases and at least a major contributory factor in the other. When the clinical data from these three cases are viewed together with that from the five other fatal cases of fat embolism occurring in sickle cell diseases gathered from the literature, it is apparent that the underlying pathological changes result in a rather consistent clinical syndrome. It is suggested

that this chain of events may be a more common cause of death in the sickle cell diseases than is generally appreciated and that they may account for some of the non-fatal cerebral and pulmonary manifestations hitherto ascribed to vascular occlusions by tangles of sickled cells.

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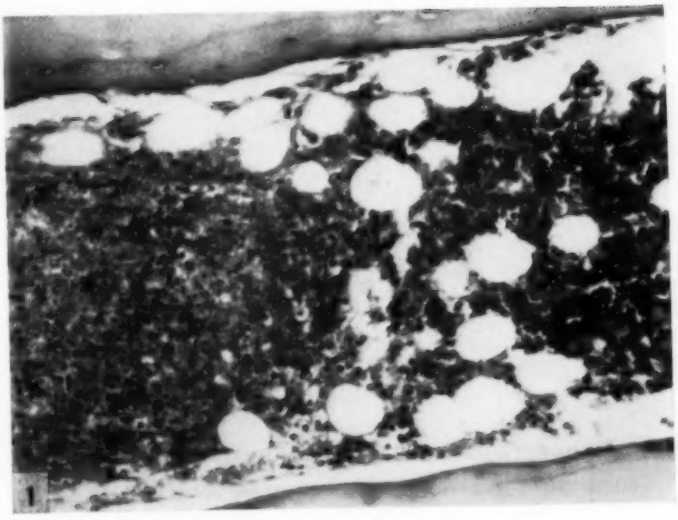
EXPLANATION OF PLATES

(Photographs by Miss Pat Whitney)

PLATE I

Fig. 1. Rib marrow from Case No. 1. The marrow on the left is necrotic, contrasting with the normal marrow on the right.

Fig. 2. A large mass of necrotic marrow containing fat is present in a large parasternal vein. Case No. 1.



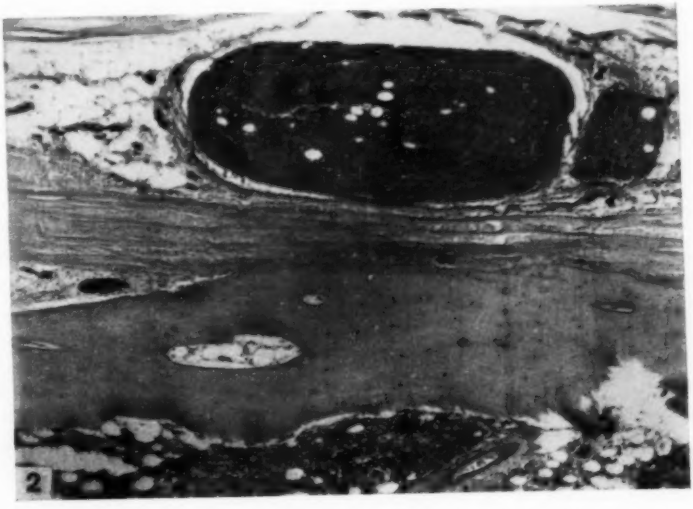
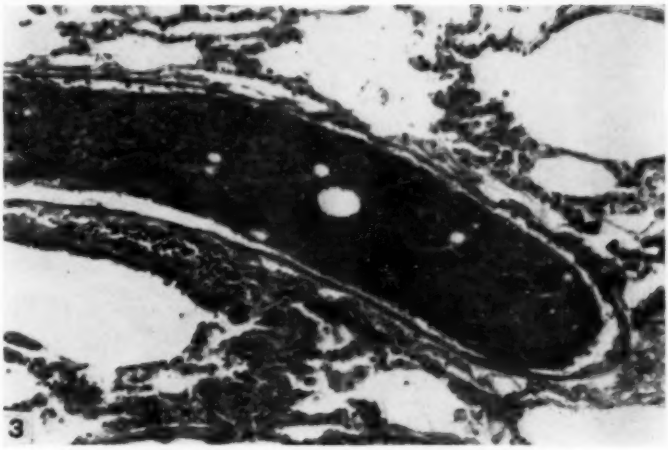


PLATE II

FIG. 3. Large necrotic bone marrow embolus. Case No. 3.

FIG. 4. Necrotic bone marrow with bony spicule taken from main pulmonary artery. Case No. 1.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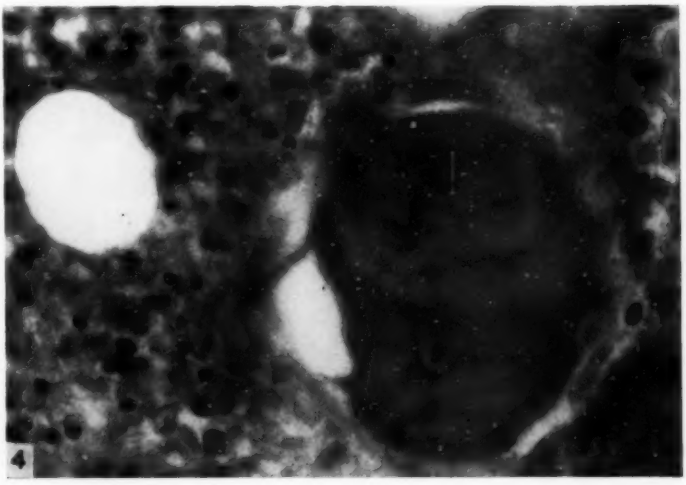
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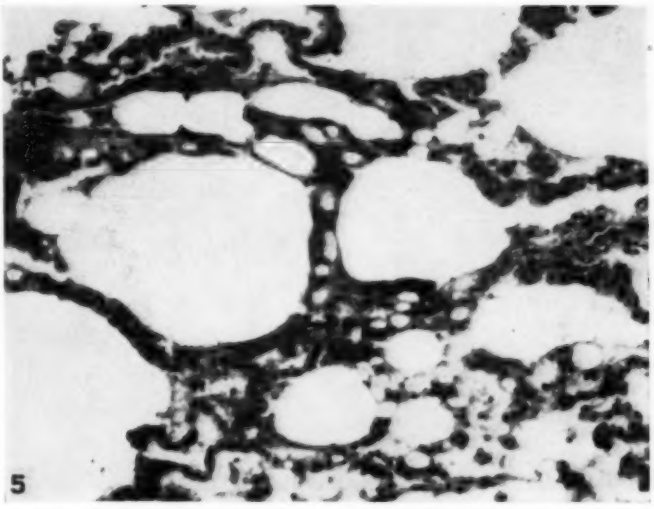
PLATE III

FIG. 5. Low power field showing numerous fat emobli in pulmonary alveolar capillaries. Case No. 3.

FIG. 6. High power of center of field seen in Fig. 5. Case No. 3.



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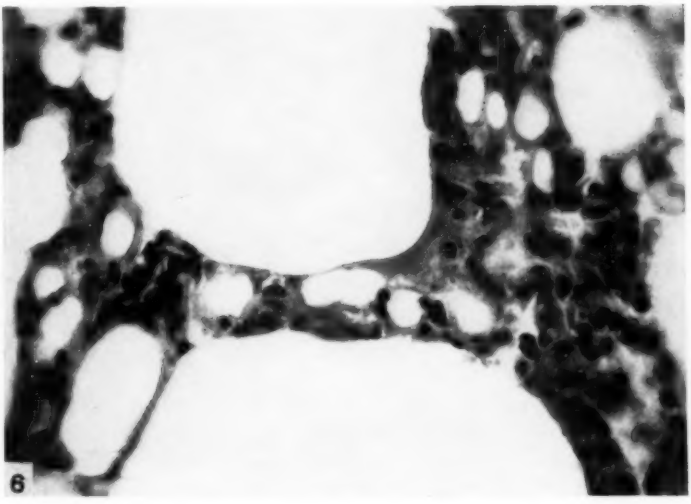

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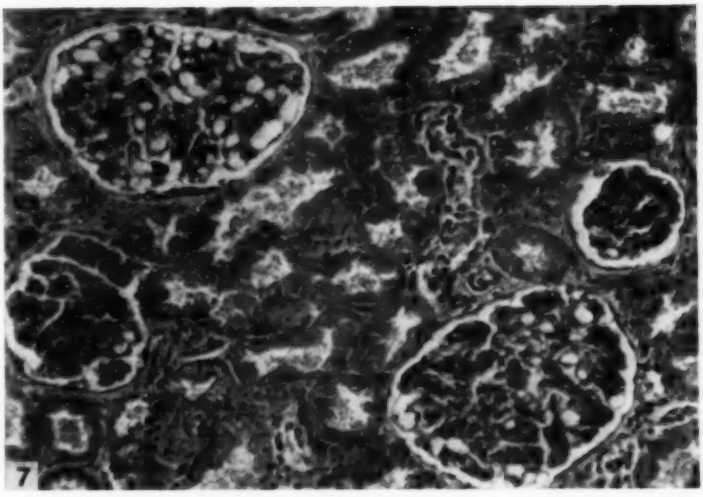
PLATE IV

FIG. 7. Fat emboli, renal glomeruli. Case No. 2.

FIG. 8. High power of glomerulus seen in upper left of Fig. 9. Case No. 2.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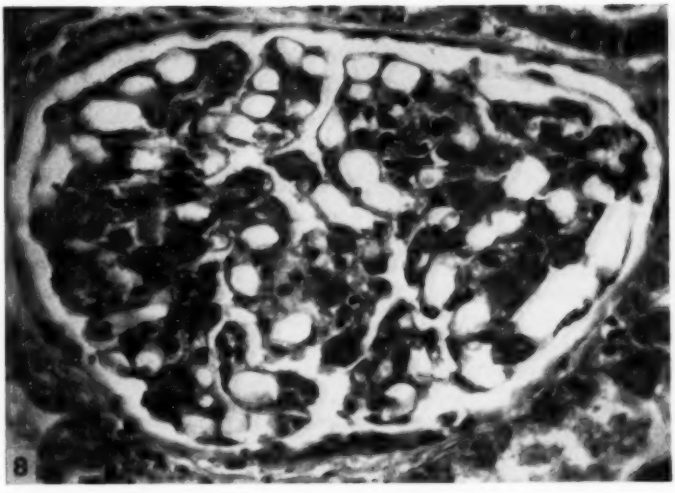
8

THE LOCALIZATION OF CIRCULATORY SHUNTS WITH INHALED KRYPTON^{85}

A Preliminary Clinical Report

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Laboratory and clinical studies, performed in the course of right heart catheterization, have demonstrated that following the inhalation of an inert foreign gas, its concentrations in arterial and right heart blood may be used to determine the presence or absence of a left-to-right circulatory shunt. The value of an inert gas for this purpose was originally demonstrated with nitrous oxide, and a comparison with the standard method of measuring oxygen differences demonstrated the distinct superiority of the nitrous oxide test (1). Although the use of cuvette oximeters (2, 3) has made possible the rapid analysis of blood samples for oxygen content, this method is still subject to the inherent errors concerned with the sampling of vena caval blood and the necessity of a steady metabolic state throughout the sampling period. It was considered that the use of a radioactive inert gas would provide the sensitivity of the nitrous oxide test, but substitute a rapid and simple counting procedure for the laborious manometric analysis necessary with nitrous oxide.

Studies with radioactive gases in experimental animals (4, 5) indicated that krypton^{85} was most suitable for clinical trial. Kr^{85} is presently being used in the catheterization laboratories of the National Heart Institute and the initial experiences with the technique form the basis of this preliminary clinical report.

MATERIALS AND METHODS

Nine patients with atrial or ventricular septal defects, one with a patent ductus arteriosus, and seven with congenital heart disease, but without left-to-right shunts, underwent right heart catheterization. The diagnosis in each instance was substantiated at operation or by other diagnostic procedures including aortography, selective angiocardiography, and indicator dilution curves.

With the tip of the catheter in the pulmonary artery, and a needle in a femoral or brachial artery, the patient was instructed to hyperventilate a mixture of 0.1 to 0.4 mc of Kr^{85} per liter of air. The Kr^{85} was administered from a spirometer, connected to the patient by means of a closed system including a mask or mouthpiece, nose clip, and a Collins three-way valve. After breathing the gas for 30 seconds, the patient was permitted to breathe room air and the exhaled air, containing the radioactive gas, was collected in a Douglas bag for the next five minutes. 2 cc. blood samples were drawn simultaneously from the catheter and the needle at

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various intervals during the 30 seconds of inhalation. The blood samples were analyzed for Kr^{85} content in an airtight chamber fixed over the window of a Geiger-Muller tube. The Kr^{85} was first extracted from the blood by the method previously described (4). The radioactivity in the systemic arterial samples ranged from 200 to 4,000 counts per minute. If the results of the pulmonary artery test indicated the presence of a shunt, the test was repeated in the right ventricle and right atrium to localize it. A blank specimen was always obtained from the catheter immediately prior to each test; its value was subtracted from that of each of the two samples. Repeat tests were performed after five minutes had been allowed for Kr^{85} desaturation.

To provide a basis for evaluating the Kr^{85} method, two other diagnostic techniques were also employed during each catheterization. Blood samples were obtained from the pulmonary artery, right atrium, right ventricle, and each vena cava. Two or three samples were drawn from each of these sites and analyzed manometrically for O_2 content (6). Nitrous oxide tests were performed by having the patient inhale a mixture of 50 per cent N_2O, 20 per cent O_2, and 30 per cent N_2 for 30 seconds as blood samples were drawn simultaneously from the needle and the catheter, which was in the same position as it had been during the Kr^{85} test. The blood samples for N_2O analysis were drawn between the 10th and 30th seconds of inhalation and were analyzed manometrically (7).

The results of the Kr^{85} and N_2O tests were described by expressing the concentration in the pulmonary arterial or right heart sample as a percentage of that in the corresponding systemic arterial sample, or PA, RV, *or* $RA/A \times 100$.

RESULTS AND DISCUSSION

The pertinent catheterization data in all patients are summarized in Table I. There were 13 Kr^{85} tests which could be regarded as controls: seven in patients without shunts, and six in patients with ventricular septal defect or patent ductus arteriosus, when the tests were performed with the catheter tip proximal to the site of the defect. In the control group, the highest Kr^{85} sample in mixed venous blood was 16 per cent, and the majority of the tests revealed pulmonary arterial or right heart samples less than 10 per cent of the systemic arterial sample. In 10 patients a total of 24 tests was carried out with the catheter tip at or distal to the entrance of a left-to-right shunt. The ratios in 22 tests exceeded 20 per cent; the other two were pulmonary artery tests of 16 and 19 per cent in patients with small atrial septal defects. The right ventricular and right atrial tests in each of these patients revealed higher values (patients 14 and 16).

The criterion for the diagnosis of a left-to-right shunt by the N_2O test has been established as a pulmonary arterial, right ventricular, or right atrial sample exceeding 20 per cent of the arterial sample. An increase in oxygen content of 1.5 vol. per cent from venae cavae into right atrium, and 1.0 vol. per cent into right ventricle or pulmonary artery is necessary for a positive diagnosis on the basis of oxygen differences.

When the Kr^{85} and N_2O tests were compared, excellent correlation was found between the results of the two tests in the same chamber. In most instances, the ratios differed by only a few per cent. In patient 12, for example, the

TABLE I

Summary of Catheterization Data in 17 Patients With and Without Left-to-Right Shunts

Patient No.	Diagnosis	Kr^{85} Tests			N_2O Tests			O_2 Samples						
		RA	RV	PA	RA	RV	PA	IVC	SVC	MVC	RA	RV	PA	A
		%	%	%	%	%	%	vol. %	vol. %	vol. %	vol. %	vol. %	vol. %	vol. %
1	Control	—	—	8	—	—	9	12.3	13.8	12.8	11.7	11.6	12.2	15.1
2	Control	—	—	0	—	—	6	—	10.2	—	11.1	10.3	11.1	16.7
3	Control	—	—	0	—	—	7	13.3	13.9	13.5	12.9	12.5	13.0	18.7
4	Control	—	—	1	—	—	0	15.5	13.3	14.9	13.6	13.2	13.6	17.9
5	Control	—	—	9	—	—	7	13.4	12.0	12.9	11.7	13.2	11.9	17.0
6	Control	—	—	5	—	—	4	12.6	13.2	12.8	13.0	12.9	12.9	18.4
7	Control	—	—	2	—	—	4	—	12.0	—	12.2	12.5	12.7	15.5
8	PDA	10	16	65	8	9	66	8.1	9.8	8.7	10.3	10.4	12.9	14.3
9	VSD	13	47	24	4	35	35	11.6	11.4	11.5	11.4	12.4	13.5	15.3
10	VSD	13	59	70	6	83	65	8.6	7.2	8.1	7.9	12.9	12.8	14.3
11	VSD	0	78	50	—	65	68	—	8.5	—	8.7	11.4	11.6	14.0
12	ASD	68	70	62	62	71	58	13.0	12.6	12.9	15.8	17.7	17.6	20.0
13	VSD	5	32	24	5	29	28	14.1	13.6	13.9	14.2	16.3	16.8	19.8
14	ASD	29	26	16	32	35	25	12.6	11.0	12.1	12.6	12.5	13.5	17.7
15	ASD	57	55	53	50	60	66	—	10.1	—	13.7	14.0	13.6	15.8
16	ASD	>100	47	19	87	47	37	15.9	13.7	15.1	14.4	14.7	14.6	18.2
17	ASD	28	21	29	33	24	31	13.5	12.5	13.2	13.6	13.8	13.9	18.6

Kr^{85} and N_2O tests are expressed as the ratio of the right atrial (RA), right ventricular (RV), or pulmonary arterial (PA) level to the systemic arterial level. The oxygen content of mixed vena caval blood (MVC) was determined by averaging the inferior vena caval samples (IVC) as twice the superior vena caval ones (SVC). Systemic arterial oxygen content (A) was determined from brachial or femoral arterial blood. In four patients, the IVC could not be entered.

greatest difference between corresponding Kr^{85} and N_2O tests was 6 per cent, while in patient 13, the largest difference was 4 per cent. Patients 14 and 16, whose pulmonary artery Kr^{85} tests were below 20 per cent, had N_2O tests of 25 and 37 per cent, a larger difference, but still in the range suggestive of a small shunt. Although precise diagnostic criteria for the Kr^{85} test have not yet been established, values similar to those established with N_2O should apply. Tests below 15 per cent are negative and those over 20 per cent are indicative of a shunt. Results between 15 and 20 per cent are considered inconclusive (8). On this basis, the correct diagnosis was established with the Kr^{85} test in all 17 patients. In three patients (8, 14, and 16), the Kr^{85} test in one chamber was inconclusive, but tests in other sites made the diagnosis certain.

A review of the oxygen determinations in Table I reveals that in 5 of the 17 patients these were misleading or inconclusive. In patient 5, the increase in oxygen content of 1.5 vol. per cent between right atrium and right ventricle was suggestive of a ventricular septal defect, but the lower content in the pulmonary arterial samples indicated either a very small defect or a metabolic change without a shunt. The Kr^{85} test was clearly negative. The increase between the

mixed vena caval and right atrial samples in patient 8 could have led to the false diagnosis of an atrial septal defect associated with the patent ductus. The increase was probably due to the sampling of laminar blood streams of low oxygen content from the inferior cava. The right atrial Kr^{85} test was negative. In patients 16 and 17, small shunts into the right atrium were not detected by oxygen differences because of the sampling of poorly mixed inferior caval blood of high oxygen content. In each instance, the correct diagnosis was made with the Kr^{85} test. In patient 14, the oxygen samples indicated that the shunt was into the pulmonary artery, but the Kr^{85} test correctly localized it to the right atrium. Again, the high oxygen content of the inferior caval samples was responsible for masking the correct diagnosis.

The limitations of the oxygen method are attributable to the variable oxygen content of mixed venous blood associated with changes in the metabolic state and to the difficulty in obtaining representative samples from the venae cavae. The inhalation of an inert gas, such as Kr^{85} or N_2O, results in a large difference between the arterial and venous levels of the gas which is independent of the metabolic state. In addition, since the inert gas content of mixed venous blood in patients without left-to-right shunts, never exceeds a fixed percentage of the arterial content (15 per cent for N_2O) it is possible to establish the presence or absence of a shunt without sampling vena caval blood. Although additional data in control patients are necessary, it appears that the diagnositc criteria for the Kr^{85} test will approximate those for N_2O. Similarly, estimation of the magnitude of a left-to-right shunt from the results of a Kr^{85} test should be possible by the application of the mixing formula described for the N_2O test (8).

SUMMARY

A method for the detection and localization of left-to-right shunts by the inhalation of a radioactive inert gas, krypton^{85}, is described. During cardiac catheterization in 17 patients with and without shunts, the gas was inhaled for 30 seconds as samples were drawn simultaneously from a systemic artery and right heart chamber or the pulmonary artery. The presence or absence of a shunt was correctly indicated in every instance by the relative radioactivity of the right heart sample. The Kr^{85} test was found more accurate than the method of determining oxygen differences and the rapid analysis of the samples with a Geiger counter made the results of the test available during the course of the catheterization.

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PROTEIN FRACTIONS OF CHICK EMBRYO EXTRACT AND THEIR GROWTH EFFECTS IN TISSUE CULTURE[1,2]

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Continuous *in vitro* propagation of certain cell strains has been achieved recently with chemically defined media free of protein components (1, 2). Supplements of serum or embryo extract added to such synthetic media can further augment the rate of cell proliferation (3). These natural products usually provide optimal growth conditions and for this reason constitute the basis of most conventional tissue culture media.

Sanford *et al.* (4) made a study of serum protein fractions prepared by the Cohn ethanol procedure in an effort to isolate a specific protein. Growth-promoting activity proved to be substantially the same in all the serum fractions, suggesting a non-specific basis for the stimulant effect.

Previous work on the protein factor in embryo extract has been confined largely to the examination of the nucleoproteins, following the initial suggestive findings of Fischer and Astrup (5). Kutsky (6) obtained an active nucleoprotein fraction by selective precipitation with streptomycin. It is indicated however, in a more recent paper of Kutsky and Harris (7), that the activity may reside in the protein moiety from which the nucleic acid has been removed.

The uncertain status of the embryo extract protein factor, especially in regard to its identification as a nucleoprotein, prompted us to approach the isolation problem with a more generalized fractionation technique. Some preliminary tests by Fischer (8) and by Baker and Carrel (9) suggested that alcohol precipitation might yield an active product. The low-temperature ethanol system of the E. J. Cohn laboratory (10), with its careful adjustment of chemical conditions, was found to be well adapted to the processing of the chick embryo proteins. In contrast to the uniform distribution of serum activity, the growth stimulant property of embryo extract was found to be localized in a definite protein fraction, precipitable at near neutral pH by a relatively low level of alcohol.

[1] This investigation was supported in part by a research grant, C-1841, from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service. In part it was aided by a grant from the American Cancer Society.

[2] A preliminary report was presented at the meetings of the Federation of American Societies for Experimental Biology. Federation Proceedings, 1957, **16:** 332.

METHODS

Embryo extract was prepared by homogenizing frozen 12 day chick embryos for one minute in a Waring blendor with an equal volume of 0.15 M ice-cold sodium chloride. After centrifugation the supernatant fluid was stored in a freezing cabinet. When thawed, a small amount of insoluble residue separated out and this was again removed in the centrifuge.

The 7.2-20 fraction, precipitated at pH 7.2 by 20 per cent alcohol, was prepared by the dropwise addition of 80 per cent ethanol at −5°C. The precipitate was redissolved in one-half the original extract volume of ice-cold 0.15 M sodium chloride and the solution was lyophilized to remove last traces of alcohol. The 5.4-20 preparation was similarly handled except for initial adjustment of pH to 5.4 with 1.0 M acetate buffer of pH 4.0. Upon resuspension of the precipitate in sodium chloride the pH was brought back to neutrality with 1.0 M disodium phosphate.

The lyophilized powder was reconstituted with Gey's balanced salt solution, modified by omitting sodium chloride, this being already present in the powder. After clarification in the centrifuge, the solution was pressure filtered through a succession of Selas porcelain filters of diminishing pore size. The final filtration, through a sterile No. 02 candle, served to sterilize the preparation. The operations were carried out at near 0°C. Protein content was determined with the biuret reaction (11). A blank reading on non-protein biuret-reacting material was obtained by applying the color reaction to the embryo extract ultrafiltrate.

Ultrafiltrate of embryo extract was prepared by pressure filtration through a Hiskey (The Emil Greiner Co.) high speed molecular filter, consisting of an extremely thin cellulose membrane mechanically supported by cotton braid.

The products were assayed in roller-tube cultures of 16 day chick embryo heart. Three cultures were implanted on the surface of a chicken plasma coagulum containing 0.1 per cent methylcellulose (Dow, Methocel, 15 cps) as a clot-wetting agent (12). The fluid nutrient medium consisted of 2 parts, human cord serum; 1 part, ultrafiltrate of chick embryo extract; 1 part, test protein in Gey's solution. The dimensions of the cultures, the major and minor diameters, were read with a measuring microscope by attaching the culture tube to a vertically oriented mechanical stage reading to tenths of a millimeter by vernier. Readings taken on the 5th day seemed best suited for comparative evaluation.

The original explants were cut approximately 2 mm. on a side, and from these there was further selection of tissue fragments for uniformity of size. A dose-response curve was obtained in a calibration experiment set up with graded concentrations of embryo extract. The area of the explant was included in the total area of the culture, this being done in the calibrating experiment as well as in the test. Fluctuations in area of the cultures arising from variations in the size of the initial explants are represented in the standard error. The errors were well within the limits required for our rough screening of the protein fractions.

RESULTS

Preliminary fractionation, Table I, gave a survey of the distribution of growth-promoting activity. The detailed schedule of the Cohn fractionation methods was not adhered to in processing the tissue extract. Table I gives the conditions for precipitation and the relative growth effects of the fractions. The most active material, fraction 1, was separated at a slightly alkaline pH with 20 per cent alcohol.

In the type of treatment where three fractions are separated successively

TABLE I

Qualitative Growth Effects of Ethanol Fractions from Chick Embryo Extract

Fraction	pH	Ethanol Vol. %	Growth Stimulation
Fraction 1	7.4	20	++++
Fraction 2	4.6	20	++
Fraction 3	4.6	40	0
Temperature −5°C.		Γ/2 = 0.15	

TABLE II

Growth Measurements on Protein Fractions from Chick Embryo Extract

Test Material	Area* of Culture mm.²	Activity of Test Solution %	Volume to Which Reconstituted	Yield %	Protein of Test Solution mg./ml.	Activity Relative to Protein %
Calibration experiment						
EE (100)†	58 ± 3.6	100	1	100	11.6	100
EE (50)	50 ± 2.8					
EE (25)	39 ± 2.3					
EE (0)	20 ± 1.9					
Measurements on protein fractions						
7.2–20	43 ± 2.5	36	½	18	5.4	78
5.4–20	44 ± 2.4	38	½	19	20.0	22

* Mean and Standard Error. 6 cultures in 2 tubes for each value. Measurements made on 5th day of growth in test medium.

† Embryo extract made up ¼ of fluid medium at maximum dosage level.

from a single batch of extract there is considerable risk of denaturation. To reduce such loss to a minimum, the procedure was altered to involve only a single exposure to alcohol. Data for these preparations are given in Table II. Fraction 7.2-20 corresponds to the above designated fraction 1; the 5.4-20 preparation, in all likelihood, includes fraction 1 together with the major portion of fraction 2.

The 7.2-20 precipitate elicited the better response relative to protein content. The 5.4-20 material, with about four times the protein, gave rise approximately to the same area of outgrowth. It should be noted however that there was some evidence of greater density of outgrowth with the 5.4-20 preparation. Plate I shows the general appearance of representative cultures grown in the absence of embryo extract and in media containing whole extract and the separated protein fractions.

DISCUSSION

The residual proteins of dialyzed serum and embryo extract were found by Fischer (13) to be inadequate for cell proliferation in the absence of certain low molecular weight accessory factors. Activity was restored by replacing the diffusible substances with an ultrafiltrate of embryo extract or with a synthetic

mixture of amino acids, vitamins, carbohydrates, and other small-molecular substances. For the critical testing of isolated proteins it is important to provide in the background medium the auxiliary non-protein nutrients. These have been supplied in our test medium by cord serum and by ultrafiltrate of embryo extract.

Another feature desired in an assay of protein fractions is insensitivity to non-specific protein effects. This can be achieved with a high background level of neutral protein in the culture medium, since this would minimize the superimposed effect of added protein as such. The cord serum, making up 50 per cent of our fluid medium, serves this purpose.

The growth stimulant effect of protein derived from embryo extract was pronounced under our culture conditions, with explanted tissue fragments on a plasma substrate. On the other hand, in replicate cultures of strain L mouse cells, grown on glass, Sanford *et al.* (14) found that the residual proteins of dialyzed embryo extract could be eliminated without appreciable reduction in potency of the culture medium.

Despite the usual success of the cold ethanol method in preserving labile proteins, no more than 20 per cent of total activity was recovered in our final product. Losses were the same whether ethanol precipitation was carried out at pH 7.2 or 5.4, making acid denaturation unlikely. Precipitation at lower ionic strength with lower concentrations of alcohol may possibly improve the yield. In terms of specific activity based on protein content, the 7.2-20 product again fell below the untreated embryo extract, registering a value of about 80 per cent. It is evident that the significance of this study does not lie in the attainment of high purification or even of good yield. It rests rather on the demonstration that the embryo extract principle can be subjected to the chemical manipulations of protein fractionation with differential concentration of activity and without prohibitive losses.

SUMMARY

Protein fractions from chick embryo extract, prepared by the Cohn low-temperature ethanol procedure, were tested for growth-promoting effects in roller-tube cultures of chick embryo heart. Activity was differentially localized in a fraction precipitated at near neutral pH by 20 per cent alcohol.

ACKNOWLEDGMENT

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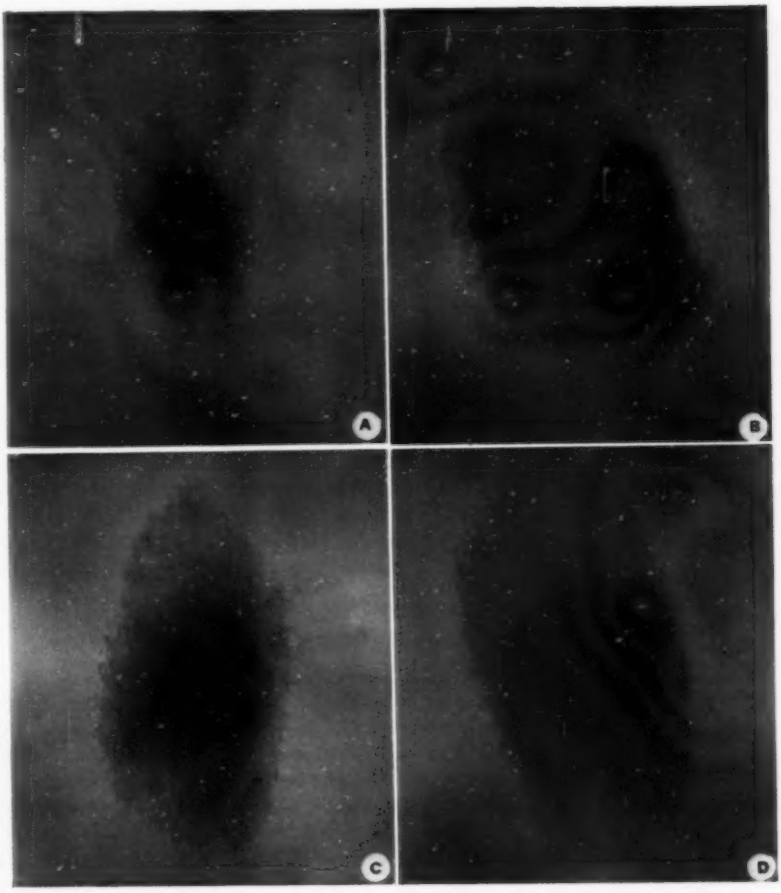
Plate I

Representative roller-tube cultures of 16 day chick embryo heart, to show extent of outgrowth at 5 days in various experimental media. Hematoxylin and eosin. × 60

A. Ultrafiltrate of embryo extract (EEUf) + cord serum.
B. Whole embryo extract + cord serum.
C. 7.2-20 fraction + EEUf + cord serum.
D. 5.4-20 fraction + EEUf + cord serum.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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY, APRIL 14, 1958

THE FAILURE OF MANUAL ARTIFICIAL RESPIRATION[1]

PETER SAFAR

Five methods of artificial respiration were studied in 25 anesthetized and curarized adults. Tidal volumes were recorded with a spirometer (oronasal mask) during performance of the manual methods and with a volumetrically calibrated pneumograph during performance of the mouth-to-mouth methods. The manual methods were studied with and without endotracheal tube, with and without oropharyngeal airway, and with and without extension at the atlanto-occipital joint (chin up, front of the neck stretched). The average tidal volumes moved by the Back-Pressure Arm-Lift method: without endotracheal tube (method taught nationwide since 1951) 126 ml.; with extension of the head 520 ml.; with endotracheal tube 655 ml. In 11 of 15 "victims" studied without endotracheal tube all tidal volumes were smaller than the estimated dead space. Similar results were obtained with the other manual methods. The spirograms suggest various types of pharyngeal obstruction due to flexion or torsion of the neck. With the mouth-to-mouth methods (without endotracheal tube), tidal volumes between 1000 and 2000 ml. were moved in all "victims" studied.

The degree and incidence of upper airway obstruction was studied in 80 anesthetized patients who were breathing spontaneously. In this study no curare drugs were used. When the neck was flexed (chin down) there was complete airway obstruction in all patients, even when an oropharyngeal artificial airway was in place, in the prone as well as the supine position. With extension at the atlanto-occipital joint only, the airway was open in 50 per cent of the patients. The other 50 per cent required in addition to extension either forward displacement of the mandible or an artificial oropharyngeal airway, or both.

THE RENAL SYNTHESIS OF BLOOD SUGAR

WILLIAM P. MCCANN AND JAMES R. JUDE

Evidence that the kidney can synthesize glucose in excess of its requirements and can deliver it into the circulating blood has been presented by other workers. Studies of glucose production by kidney slices *in vitro* and measurements of renal arteriovenous differences for glucose have been described. It is difficult, however, to interpret the physiological significance of these previous reports for several reasons. For example, all previous studies have employed glucose assay procedures which lack optimal specificity and precision. Furthermore, experiments in whole animals have been done in the presence of anesthesia, acute operative trauma, ureteral ligation, and in some cases hepatectomy or evisceration.

In order to re-evaluate this phenomenon, glucose concentrations have been measured in the blood and plasma of the renal vein and aorta in calm, unanesthetized dogs in whom plastic catheters were implanted in these vessels at a prior operation. The time period between the initial operation and the definitive experiments ranged from 2 days to 3 weeks. Glucose assays were performed by a method which depends on the enzyme glucose oxidase. The surgical and analytical techniques were described.

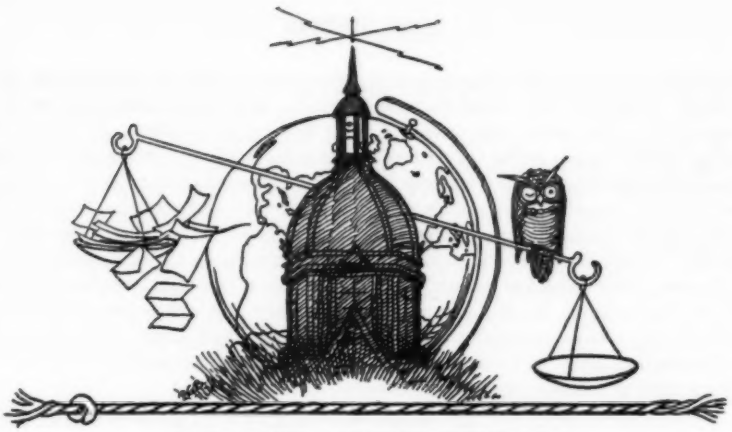
The data obtained indicate that in the unanesthetized dog, previously fasted for 16 to 18 hours, the glucose concentration of the renal vein blood is almost always higher than that

[1] This study was supported by the Research and Development Division of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-858.

in the aorta. In eight dogs, the mean plasma increment was +1.9 ± .46 mg/100 ml. In one dog in whom both whole blood and plasma increments were measured, the mean whole blood increment was +.97 ± .202 mg/100 ml, and the corresponding plasma increment +1.49 ± .212 mg/100 ml. In two dogs, catheters were also placed in the inferior vena cava, where the plasma glucose concentrations were consistently lower than in the aorta.

The increments in glucose concentration seen in the renal vein cannot be explained by hemoconcentration, which could account for an increment of only .2 or .3 mg/100 ml. The data obtained are interpreted to indicate a renal glucose production of 9.5 to 18.5 mg/kg body weight/hour which is estimated to be within the broad range of 4 to 13 per cent of the hepatic glucose output.

It is suggested that this renal glucose production is not specifically directed towards the regulation of the blood glucose concentrations, but that it may have a bearing on some of the intrinsic activities of the nephron.



BOOK REVIEWS

A Manual of Paper Chromatography and Paper Electrophoresis, 2nd edition. By RICHARD J. BLOCK, EMMETT L. DURRUM AND GUNTER ZWEIG. 710 pp., $12.80. Academic Press, New York, N. Y.

The second edition of this very comprehensive manual on paper chromatographic and paper electrophoretic methods has added a good deal to the material included in the first edition of only three years ago. The number of papers in the literature of chromatography increased constantly for awhile, but it would seem that the acceleration rate is now decreasing. In comparing the material of this volume with the first edition, one finds that there are a few additional explanations provided for the mechanism involved in chromatographic or electrophoretic separations. The new material is of necessity restricted largely to an exposition of variations in methods and the use of solvent systems to substances being separated.

In the first edition of this book, I believed and I found company with other reviewers, that Durrum's section on paper electrophoresis seemed a poor stepchild to the main body of chromatographic material. This situation is not greatly corrected in the present edition. More critical evaluation of comparative results of electrophoresis, of serum, for example, with different methods of paper electrophoresis, and a more critical and complete discussion of the problems involving quantitation seem indicated.

As must occur with textbooks in a rapidly expanding field such as this, the books are hardly in press before they are out of date in certain respects. The use of agar gel electrophoresis, for example, is becoming increasingly useful, but is considered in the barest detail in this book. Overall, this text should continue to be a useful reference source but is not without its faults of omission.

ERNEST W. SMITH

Occupational Diseases of the Skin, 3rd edition. By LOUIS SCHWARTZ, LOUIS TULIPAN AND DONALD J. BIRMINGHAM. 381 pp., $18.00. Lea & Febiger, Philadelphia, Pa.

In our dermatologic library is an extremely worn copy of the second edition of this book. This appearance gives mute testimony to the fact that the reference source of occupational dermatoses has almost constant use in the practice of dermatology as well as of medicine in general.

The book is written in a very readable fashion which is a notable achievement itself, in

view of the fact that so many hundreds of individual agents and the resulting damages are considered. There are very useful chapters listing the appropriate concentrations of substances to be used in patch tests, covering nearly 700 such preparations. Extensive lists of irritating plants, waxes, resins are particularly valuable sources of reference. The book is divided, as in previous editions, into chapters concerning hazards of radiation, dermatoses caused by acids, by alkalis, by metals and those caused by hydrocarbons. Synthetic dyes, wearing apparel, cosmetics and detergents are each handled individually. Insecticides and fungicides, the number of which has been increased enormously, the dermatoses of leather manufacture, and varnish and lacquer, solvents and irritant plants and woods are discussed. And, in a final chapter, the skin hazards of each of many occupations listed alphabetically provides a very useful cross-reference of the material. This book meets all the criteria for a valuable textbook. It is pertinent, readable, to the point and completely encylopedic concerning a topic of common and constant interest.

ERNEST W. SMITH

Clinical Electrocardiography. The Spatial Vector Approach. By ROBERT P. GRANT. 225 pp., $7.50. McGraw-Hill Book Co., New York, N. Y.

The prospective reader of this book should not be turned away by the phrase "spatial vector approach" which appears in the title. This book is not concerned with the vector loops recorded by complex lead systems and cathode ray oscilloscopes. Dr. Grant utilizes the vector approach to present a sensible, flexible, physiological method for interpreting the conventional electrocardiogram. In the author's words, "An electrocardiographic deflection is, after all, simply a measurement of components of electrical forces, and therefore the most rational and objective method for handling this information is to express it in the form of directed electrical forces or vectors." There can be no question about the truth of this statement and it is a pleasure to see all the isolated bits of clinical electrocardiographic lore fit neatly into a unified concept.

The vector approach emphasizes the fact that the different leads merely view the same phenomenon from different vantage points. This concept of interpretation is unquestionably superior to the older method based on patterns and contours in individual leads. A simple presentation of QRS and T vectors on the conventional triaxial frontal plane reference system is used throughout the book. This display of information can be produced from the conventional leads in a few seconds. Unfortunately, however, there are still rules and laws which must be remembered to separate the normal from the abnormal records. It is not easy for one experienced in older methods to convert to "vector thinking" and as the author points out, probably not worthwhile in most instances. In difficult problems such as myocardial infarction in the presence of bundle branch block and the so-called "abnormal T wave", it is undoubtedly worthwhile to draw out the vector pattern. In these two cases the vector method may be useful in separating normal and abnormal records where inspection alone is often inconclusive.

The book will provide a healthy introduction for the student, but will probably need to be supplemented by a book covering the practical problems of interpretation in the terms used by the majority of today's electrocardiographers. Established electrocardiographers will enjoy and profit from this book and will realize that the vector approach offers a more nearly accurate interpretation of electrophysiological events and is, therefore, certain to become more popular and useful.

RICHARD S. ROSS

The Clinical and Immunologic Aspects of Fungous Diseases. By J. WALTER WILSON. 280 pp., $6.75. Charles C Thomas, Springfield, Ill.

This small volume is designed to supplement existing textbooks of mycology by presenting a detailed account of the clinical and immunologic aspects of fungous diseases of man. The author's objectives are achieved in part, for the immune response to mycotic infection is described clearly and completely, and these chapters make up the most interesting and instructive portions of the book. However, the clinical manifestations are considered only briefly and unfortunately, rather superficially, and add little to existing texts.

Persons with more than a casual interest in mycotic diseases will find the book enjoyable, possibly stimulating, because the author has ended a period of "conservative waiting" by presenting "theory and concept" formulated during 25 years of practice in this field.

EDWARD W. HOOK, JR.

Medical Radiation Biology. By FRIEDRICH ELLINGER. 945 pp., $20.00. Charles C Thomas, Springfield, Ill.

This is a monumental compendium of information concerning the biologic and medical effects of electromagnetic and corpuscular radiations. It includes a surprising amount of detail, along with references to reviews as sources of full detail wherever possible. It is well organized, and is integrated as befits the author's stature in the field. Dr. Ellinger, who is Chief Radiation Biologist and Head of the Pharmacology Division, Naval Medical Research Institute, has put all of his vast knowledge and experience into his book. Its scope is encyclopedic.

It would be of great value for its bibliography alone. 4600 references, many going back to the pioneer days of radiation physics, are ably cross-indexed and used to excellent advantage in documenting the text. For example, the law of Grotthuss and Draper, stating that only absorbed radiant energy is capable of inducing physical or chemical changes in matter, seems so obvious today as scarcely to need support. The book cites the original and confirmatory publications, in 1819 and 1841–45. There is similar meticulous documentation for most points discussed in the text.

Many nice and careful distinctions are made, such as the difference between radioresponsiveness and radioresistance. It is not generally appreciated that although the lymph nodes are responsive to relatively small amounts of radiation, they are quite radioresistant, regenerating after exposure to large doses. On the other hand, broad generalizations are occasionally made on inadequate evidence, and lapses into ambiguity are not rare.

One of man's greatest problems is to accept and live within his limitations. As the author points out in his preface, for one man to attempt a book of this vast scope is fraught with dangers, some of which he proceeds forthwith to demonstrate. His point of view is valid, whether or not held universally, but his work suffers from lack of editing. It contains many errors of fact, some undoubtedly careless and others probably results of confusion in a welter of detail. The dermatologists will scarcely agree that ". . . the skin is only 1 cm. thick of which the basal layer averages between 0.5 and 1.0 mm. . ." Failure to distinguish between total body irradiation and exposure of a localized field can be misleading, as can failure to take adequate cognizance of differences in quality as affecting depth-dose and, consequently, biologic effect. In a work of this magnitude, done by one man, such errors can be condoned, but they are inevitably disturbing and they may leave questions about the degree and nature of the error.

The book is badly in need of other proof-reading. It is not in the author's native tongue, and frequently reads as though translated literally from another language. Errors in spelling,

which abound, are usually merely annoying, but flaws in grammar and punctuation are sometimes so bad as to obscure the author's meaning. This is unfortunate, for what he has to say is well worth lucid presentation. Such errors could have been obviated so easily that it is difficult to forgive the publisher for not having seen to them.

The author's wish to be all things to all people is understandable, but somewhat ambitious. The work presupposes at least a passing acquaintance with radiation physics, and may therefore be a little beyond the scope of the average physician. The radiologist, the dermatologist, and the radiation biologist, however, will find it invaluable for many reasons, not least its organization of the subject.

The paper is good, the type clear and legible. The table of contents is amply detailed. The use of capitals to emphasize points whose importance eludes the reader may become annoying.

Faults notwithstanding, this is a colossal contribution to a vast and complex field. To this reviewer, the work seems destined to be the great fount of information, the starting point of every review, and the foundation of every well-documented paper to come in this entire field.

MARK HOLLANDER

Schedules of Reinforcement. By C. B. FERSTER AND B. F. SKINNER. 741 pp., $6.50. Appleton-Century-Crofts, New York, N. Y.

Experimental psychologists are appearing more and more frequently in medical research settings. A powerful impetus to this collaboration has been the search for ways of assaying and studying drugs that are aimed at the modification of behavior. But the methods and concepts of the experimental psychologist are pertinent to a broader range of problems in medical research than just those in psychopharmacology. In a volume that even before its publication was certain to achieve the status of a handbook, Ferster and Skinner demonstrate the potency of some of these methods and concepts in the study and control of behavior.

Schedules of Reinforcement is the most recent statement of the position to which B. F. Skinner, Edgar Pierce Professor of Psychology at Harvard, has swung a large number of American experimental psychologists. Partly, this is due to the success he has had in applying his techniques to the control of behavior, partly to disillusionment with other programs. The book itself reports five years of intensive research in collaboration with C. B. Ferster.

Skinner's approach to the study of behavior can be illustrated by the experimental situation that is the basis for nearly all of the data reported in the book. This is one in which a pigeon, in an enclosed box, pecks at a translucent key to obtain access to grain for a few seconds. The frequency of responding, i.e., pecking, is taken to be the basic datum in the description of behavior. This frequency is affected by a number of variables, including, of course, hunger; but Skinner is mainly interested in the effect of reinforcement schedules—the correlations between responses and reinforcements. The latter are defined as events which increase the frequency of responding. Usually, the reinforcement is the presentation of food, but it can be a variety of other things as well, such as the withdrawal of a noxious stimulus.

The pigeon can be allowed access to food every time it pecks the key. But reinforcement does not have to be delivered each time. We may, for instance, require the bird to make a pre-determined number of responses (either fixed or variable) to obtain food. This is a *ratio* schedule. Or, we may deliver a reinforcement for the first response made after a specified period of time, again either fixed or variable. This is an *interval* schedule. By exploiting these two basic parameters of a reinforcement schedule (time and number), one can devise schedules that are extremely complex and in which behavior is maintained even though reinforcements occur intermittently. Each schedule produces a characteristic effect on the frequency and temporal distribution of responses.

The book is arranged in chapters corresponding to the various kinds of schedules that Ferster and Skinner have investigated. Studies of the effects of drugs and brain lesions are also reported. In one of these experiments, the bird worked on a variable interval schedule and was reinforced with grain infused with phenobarbital. When enough of the drug had been consumed so that the rate fell below a certain value, the reinforcement was switched to clean grain until the rate reached the previous level, then switched back to the drug-infused grain. This was an attempt to establish a behavioral criterion for repeated administration of a drug rather than the criteria commonly used. While it did not turn out to be completely successful, the experiment illustrates how Skinner's methods may be extended to a wide range of problems that involve behavior.

The arrangement of the book reflects the thorough empiricism of its authors, for in it one finds no attempt to formally order the data by some sort of scheme or model. This feature makes it difficult for the reader unacquainted with Skinner's work to grasp exactly what the authors are trying to do. But if one realizes that their aim is an exhaustive catalog of how reinforcement schedules shape and control behavior, then even the nonpsychologist reader can read it with profit, for it mirrors just what is happening on some of the most vigorous frontiers of behavior science.

BERNARD WEISS

Oral Medicine. Diagnosis and Treatment, 3rd edition. By LESTER W. BURKET. 558 pp., $14.00. J. B. Lippincott Co., Philadelphia, Pa.

Dr. Burket's wide experience in both Medicine and Dentistry has helped him accomplish the difficult task of describing the relationships between oral and systemic diseases. The book is a comprehensive, well organized, significant and thoroughly practical, well written treatise. The general format of this, the third edition, is unchanged. Increase in knowledge about diseases affecting the oral cavity and progress in therapy necessitated complete rewriting of many chapters and revision of others. The sections devoted to illnesses of major clinical importance have been expanded. The bibliography has been revised. Key references of current research and therapeutic developments have been added.

The second section of the book deals with more localized manifestations of illnesses on gingivae, gums, lips and oral mucosa. It includes metal intolerance, intoxication and allergic manifestations. The third section is devoted to diseases of the tongue and dermatologic diseases of dental interest. In other sections are discussed: diseases of the organ systems; nutritional deficiencies and metabolic disorders; specific infectious granulomata; focal infection, dental pediatrics and Gerodontics; occupational hazards in dentistry and industry. Section nine, written by S. Gordon Castigliano, is devoted to oral cancer and discusses its general aspects: diagnosis, differential diagnosis, localization, incidence, general principles of treatment, etc. A small color atlas and a regional diagnostic index form the last two sections of the book. Many illustrations in black and white are in life-size. They are of excellent quality and of great value in clarifying certain points in the text. Extensive bibliographies appear at the end of each chapter.

The paper, printing, and manner of presentation make this book a delight to read. Between its covers the dentist and physician will find much valuable information and assistance enabling him to carry the large share of responsibility for his patients' health. The present edition should, even more than the previous editions, serve as a basic work in oral medicine. It can be recommended with enthusiasm for medical students, dentists and physicians, also for reference libraries.

GEORGE T. NAGER

BOOKS RECEIVED FOR REVIEW

The Brain and Human Behavior. (Research Publications of the Association for Research in Nervous and Mental Disease, Vol. 36). Edited by HARRY C. SOLOMON, STANLEY COBB AND WILDER PENFIELD. 564 pp., $15.00. The Williams & Wilkins Co., Baltimore, Md.

Ciba Foundation Symposium on Chemistry and Biology of Mucopolysaccharides. Edited by G. E. W. WOLSTENHOLME AND MAEVE O'CONNOR. 323 pp., $8.50. Little, Brown & Co., Boston, Mass.

Group Processes. Transactions of the Third Conference Oct. 7, 8, 9 and 10, 1956. Edited by BERTRAM SCHAFFNER. 328 pp., $4.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

A Laboratory Guide in Chemistry, 3rd edition. By JOSEPH H. ROE. 244 pp., $2.50. The C. V. Mosby Co., St. Louis, Mo.

The Medical Management of Cancer. By HENRY D. DIAMOND. 179 pp., $6.75. Grune & Stratton, New York, N. Y.

Polysaccharides in Biology. Transactions of the Second Conference April 25, 26 and 27, 1956. Edited by GEORG F. SPRINGER. 245 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

Principles of Research in Biology and Medicine. By DWIGHT J. INGLE. 123 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa.

Psychiatric Nursing. Syllabus and Workbook for Student Nurses, 2nd edition. By LENORE KIMBALL. 186 pp., $3.00. The C. V. Mosby Co., St. Louis, Mo.

Psychoprophylactic Preparation for Painless Childbirth. By ISIDORE BONSTEIN. 143 pp., $2.50. Grune & Stratton, New York, N. Y.

Self-Teaching Tests in Arithmetic for Nurses, 5th edition. By RUTH W. JESSEE. 137 pp., $2.40. The C. V. Mosby Co., St. Louis, Mo.

Sherrington, Physiologist, Philosopher and Poet. By THE RIGHT HONOURABLE LORD COHEN OF BIRKENHEAD. 108 pp. Liverpool University Press, Liverpool, England.

Tuberculosis in White and Negro Children. Vol. I, The Roentgenologic Aspects of the Harriet Lane Study. By JANET B. HARDY. 122 pp., $7.50. Published for the Commonwealth Fund by the Harvard University Press, Cambridge, Mass.

Tuberculosis in White and Negro Children. Vol. II, The Epidemiologic Aspects of the Harriet Lane Study. By MIRIAM E. BRAILEY. 103 pp., $4.50. Published for the Commonwealth Fund by the Harvard University Press, Cambridge, Mass.

Ward 4. By JAMES HOWARD MEANS. Foreword by CHARLES SIDNEY BURWELL. 187 pp., $4.50. Harvard University Press, Cambridge, Mass.

CHRONOTROPIC RESPONSES TO SYMPATHOMIMETIC AMINES IN EXPERIMENTAL COMPLETE HEART BLOCK: THE INFLUENCE OF PENTOBARBITAL, HYDROCORTISONE, AND ACID-BASE CHANGES[1]

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The treatment of Stokes-Adams disease is frequently unsuccessful. The voluminous literature devoted to this disorder includes few attempts (1, 2) to clarify therapeutic problems through the study of laboratory animals. This is surprising since procedures for creating chronic complete heart block in dogs were described by Erlanger (3) more than fifty years ago. Such preparations are well suited for the study of Stokes-Adams disease for several reasons. The course of the experimental disease often resembles the acquired human condition and the animals are free of arteriosclerosis and intrinsic disease of conducting tissue.

For these reasons we began the study of pharmacologic reactivity of ventricular pacemakers in dogs with surgically produced complete heart block. Our first objective was to determine the relative effectiveness of isoproterenol, l-epinephrine and levarterenol as chronotropic agents. Our second objective was to attempt to modify such responses by a variety of substances having experimental or clinical value. The influence of pentobarbital was studied as a necessary preliminary to experiments which required anesthesia. Adrenal cortical steroids have been described as clinically useful in Stokes-Adams disease (4), and their effects upon sensitivity to sympathomimetic amines were consequently of interest. The use of hypertonic solutions of sodium lactate in patients with complete heart block has produced conflicting reports. Bellet, Wasserman and Brody (5, 6) have favored the use of this agent as a safe and effective means of increasing ventricular rate. Others (7) have observed hazardous arrhythmias following its administration. The mechanisms whereby rate is accelerated and arrhythmias produced remains obscure. Consequently the effects of alkalosis were studied in the dog in an effort both to resolve conflicting observations and to clarify the mechanisms involved. Acidosis is known to produce atrial slowing (8, 9). The degree of such slowing is usually insufficient to have clinical importance. However, when heart rate is initially slow, as it is in complete heart block, any further slowing decreases cardiac output (10).

[1] This study was supported in part by Research Grant HF-6308-C of the United States Public Health Service.

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It seemed desirable therefore to assess ventricular slowing associated with acidosis. Finally, certain patients with Stokes-Adams disease become refractory to sympathomimetic amines. The observation by Houle and colleagues (11) of reduced peripheral vascular responsiveness in acidosis suggested that ventricular pacemakers might also be less sensitive in acidosis.

METHODS

Experimental animals. Complete heart block was produced by the method of Starzl and Gaertner (12) in mongrel dogs of both sexes by incising the bundle of His at a point 5–10 millimeters anterior to the coronary sinus. Gaertner suggested (13) that a number 11 Bard-Parker blade be used to dip beneath and prick the A-V bundle in an upward tenting motion. This maneuver facilitated the production of heart block. Complete block was persistent until time of death in twelve animals weighing 7 to 15 kilograms. All animals were allowed to recover from surgery for at least five days, usually several weeks, before beginning study of drug responses.

General experimental procedure. Sympathomimetic amines[4] were isoproterenol hydrochloride (Isuprel,® isopropylarterenol), l-epinephrine bitartrate, and levarterenol bitartrate monohydrate (Levophed,® l-norepinephrine). A stock solution of each drug containing 0.25 micromols per milliliter was prepared by dissolving appropriate amounts of powdered drug in 0.9 per cent sterile saline containing 2 milligrams of sodium bisulfite per milliliter. The stock solution was continually kept iced and always used within nine days of preparation. Further dilutions from the stock solution were prepared with saline-bisulfite diluent either immediately before use or, in cases where the same dose was repeated, at least every ninety minutes and kept on ice in the interim.

Drug dilutions were given intravenously within one to three seconds in a volume of 0.1 milliliter per kilogram of body weight. Such injections were immediately followed by a rapidly given wash of 1.5 milliliters normal saline containing approximately five units of heparin per milliliter. Occasional injections of 1.5 milliliters of saline were given alone as controls.

In order to prevent pain and excitation with drug administration all agents were given through polyethylene tubing whose internal tip was in one of the venae cavae close to the right atrium. Once such a catheter was in place its position was not disturbed during the remainder of the day.

Electrocardiograms, usually from one of the standard limb leads, were recorded on a Sanborn Viso-Cardiette. The most reliable estimate of ventricular rate was found to be the mean of ten consecutive R-R intervals immediately preceding drug injection. Atrial resting rate was taken as the mean of ten or twenty consecutive P-P intervals.

Respiratory methods. When artificial respiration was required a cuffed endotracheal tube was inserted and ventilation maintained by an intermittent positive pressure respirator described by Hanlon and coworkers (14). The respirator was modified to give a pressure curve with a smooth gradual inflation phase, a more rapid early deflation phase, and a prolonged post-deflation phase. Inflation pressures of 12 to 14 millimeters of mercury and respiratory rates of 14 to 18 per minute were employed.

Chemical methods. Hydrogen ion concentration was estimated by a Beckman, model G, pH meter and a Beckman blood electrode assembly, 290–31. The electrode assembly was

[4] Generously supplied by Dr. A. M. Lands of the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

kept immersed in a water bath at 37.0 ± 0.5 degrees centigrade. Determinations were made on 2 milliliter samples of caval or arterial blood. Samples of caval origin were always drawn into heparinized syringes from the vena cava opposite to that receiving alkali or acid fusions. When arterial blood was used animals were given intravenous heparin, 4 milligrams per kilogram, before beginning procedure. Samples were handled anaerobically and introduced into the electrode assembly within approximately forty-five seconds of beginning withdrawal from an inlying polyethylene tubing. pH readings were made two minutes after filling the assembly. Standard buffer solution pH estimations, made at the beginning and the end of experiments, agreed within 0.03 units.

Arterial plasma for estimation of potassium concentration was obtained by repeated centrifugation of 2 milliliter quantities of blood. Plasma was sealed and refrigerated until potassium concentration was measured by a Patwin, model FC, flame photometer (15).

Statistical analysis. "Student's t test" was used to determine significance of certain differences and changes in response; "t" values were obtained by the method of paired comparisons of appropriate data (16).

PROCEDURES AND RESULTS

Relation of Resting Ventricular Rate and Response to l-epinephrine and Levarterenol

Three dogs were trained to lie quietly throughout the procedure and occasionally fell asleep. The only restraint was a lightly applied muzzle to prevent licking which caused interference with recordings.

The *change* in ventricular rate produced by repeated injections of 0.0045 micromols per kilogram l-epinephrine and levarterenol was found to vary widely and was inversely related to control rate (Fig. 1). There was no correlation between the relatively fixed *maximum* or *absolute* response and the slightly variable control rate (Fig. 2). Similar results were obtained in the two other dogs examined.

Chronotropic Effects of Sympathomimetic Amines

A. *Ventricular responses to a single size dose*

When maximum responses to 0.0045 micromols per kilogram l-epinephrine and levarterenol are compared in unanesthetized dogs levarterenol was always significantly superior ($p \leqq 0.02$) as demonstrated in Table I. In two animals the duration of response was measured and found to be longer after levarterenol than after l-epinephrine. In one animal (488) the interval from start of response to return of control rate was forty-three (standard error of mean: ±4.1) seconds for levarterenol and twenty-eight (±2.0) seconds for l-epinephrine. In the other animal (412) mean duration of levarterenol response was eighty-eight seconds (±1.9) while l-epinephrine mean duration was forty seconds (±2.9). In every case these are the means of at least seven values.

When followed for several months two of three animals exhibited a gradual decrease in resting rate and a lesser decrease in absolute responsiveness to l-epinephrine and levarterenol.

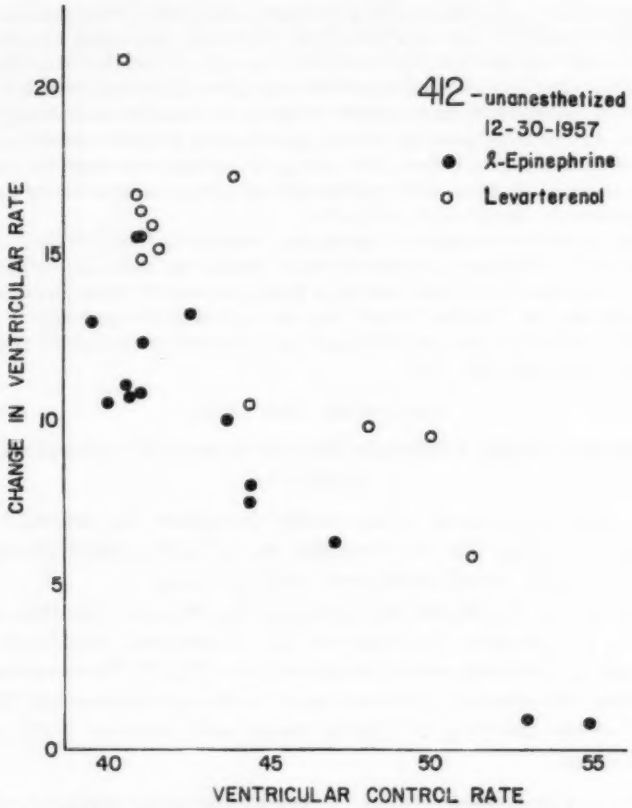

FIG. 1. Relation between control (resting) ventricular rate and change in rate produced by drugs: single day observations. All rates in this and following figures are given in beats per minute. Drug dosage is 0.0045 micromols per kilogram body weight. All observations in one animal.

B. *Ventricular responses to doses of varying size*

Procedure. Ventricular chronotropic responses to isoproterenol (0.003, 0.005, 0.001, 0.002, and 0.0045 micromols per kilogram body weight), l-epinephrine (0.002, 0.0045, 0.007, 0.015, and 0.025 micromols per kilogram), and levarterenol (same dosage as indicated for l-epinephrine) were determined on each of five different days during a month's time in three trained unanesthetized dogs. At least six minutes elapsed after the three smallest doses of each drug before injecting the next dose. Ten or more minutes elapsed between trials of the two largest doses of each drug. Comparable amounts of each drug were given in a random fashion. It was feared that the two largest amounts

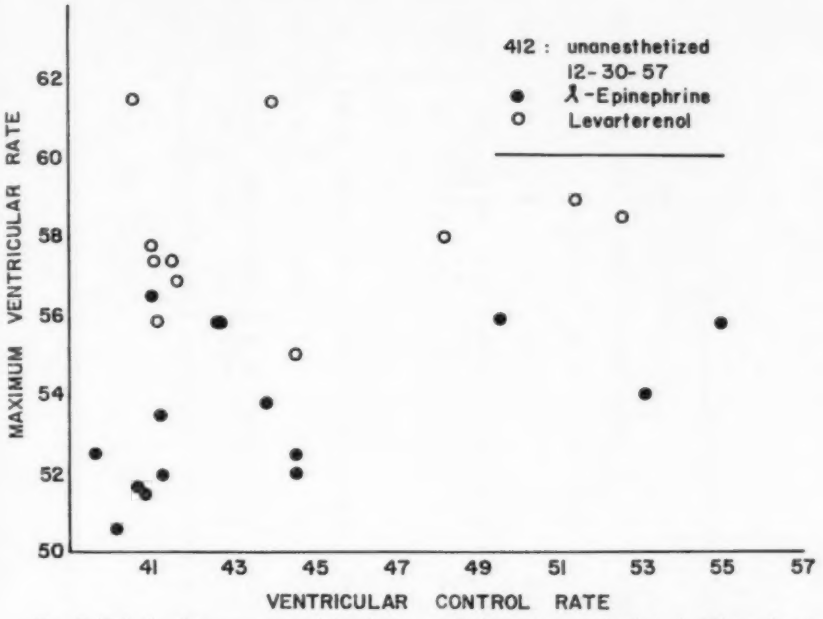

FIG. 2. Relation between control (resting) ventricular rate and absolute (maximum) rate produced by drugs: single day observations. Data from the same experiments as in Figure 1.

of each drug might have a cumulative effect and accordingly these were always the last given.

Results. The absolute (maximum) ventricular responses to five different doses of isoproterenol, l-epinephrine, and levarterenol are given in Figure 3. Isoproterenol was far superior to levarterenol. From Figure 3, showing the pooled means of absolute responses, a rough estimate indicates that approximately six times as much levarterenol as isoproterenol is needed to produce the same ventricular chronotropic effect throughout the dose range studied. By this same method of approximation levarterenol appears to be approximately twice as potent as l-epinephrine. The relatively greater effect of levarterenol was usually highly significant ($p < 0.02$) by the method of paired individual comparisons in two of three dogs. Indeed the response to levarterenol was greater than to l-epinephrine in 68 out of 74 paired comparisons. The same order of results is found when changes in rate rather than absolute maxima are compared. Since change in rate is a function of resting rate variance is greater with this parameter than with maxima and the "t" values for the differences between l-epinephrine and levarterenol correspondingly less. Ectopic beats developed once in forty-five isoproterenol trials, once in forty-four l-epinephrine trials, and six times in forty-five levarterenol trials.

TABLE I

Maximum Ventricular Responses to Levarterenol† and l-epinephrine† in Unanesthetized Animals*

Dog	412					488			549				
Date	11–11	11–22	11–29	12–20	12–30	11–4	11–8	12–6	11–6	11–13	11–29	12–6	12–18
L max	63.9	59.0	56.6	59.6	57.8	56.2	54.0	49.2	65.6	64.7	67.2	64.2	71.5
	0.9‡	0.9	1.6	1.7	2.0	2.0	0.9	1.9	0.5	0.7	1.3	0.8	3.1
	(4)§	(8)	(4)	(5)	(13)	(4)	(7)	(4)	(4)	(8)	(5)	(4)	(6)
E max	56.8	53.3	51.3	52.4	53.4	52.2	48.3	43.5	59.0	57.8	59.3	57.4	63.3
	0.2‡	0.8	1.9	1.5	1.9	1.4	0.8	1.2	0.2	0.9	2.0	1.0	0.4
	(4)§	(8)	(4)	(5)	(14)	(4)	(7)	(4)	(4)	(8)	(5)	(4)	(5)
L max - E max	7.1	5.7	5.3	7.2	4.4	4.0	5.7	5.7	6.6	6.9	7.9	6.8	8.2
p‖	<0.001	<0.001	<0.01	<0.001	<0.001	<0.02	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
Control Mean	36.8	39.3	35.5	41.2	44.0	40.3	35.5	29.2	37.8	38.6	37.7	38.0	42.0
Control Range	35.0–39.1	37.8–40.5	33.6–37.2	39.1–42.7	39.6–55.0	39.3–41.5	32.8–38.7	25.8–31.8	35.4–40.7	35.0–42.7	32.0–42.6	35.2–42.4	37.6–45.2

* In this and succeeding tables heart rates are given in beats per minute and maximum rate refers to absolute response without regard to pre-existing control or resting rate.

† 0.0045 micromols per kilogram body weight.

‡ Standard deviation.

§ Number of determinations.

‖ Significance of difference between levarterenol (L max) and l-epinephrine (E max) responses in this and succeeding tables.

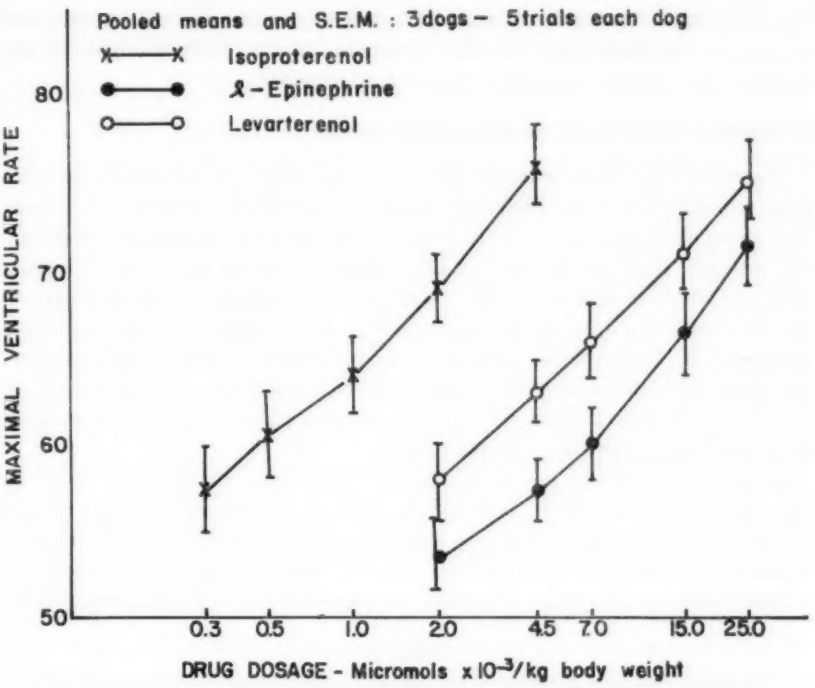

FIG. 3. Log dose-response relationships. Each point represents the mean of fifteen determinations. Maximum rates represent absolute values without regard to resting rate Bar at each point represents standard error of mean.

C. *Ventricular responses to continuous infusion*

Procedure. Three unanesthetized trained dogs were studied. Constant volume infusions, 0.2 to 0.4 milliliters per minute, of isoproterenol (0.00015 micromols per kilogram per minute), of l-epinephrine (0.0015 micromols per kilogram per minute), and of levarterenol (0.0015 micromols per kilogram per minute) were individually given for four minutes at the end of which time the infusion rate was doubled. Infusion was stopped after a total of seven minutes. At least fifteen minutes elapsed between trials of different drugs. After completing all infusions the procedure was repeated in two dogs. In a third dog (488) the amounts infused were doubled rather than repeated in order to produce an appreciable ventricular response.

Results. Ventricular chronotropic sensitivity to continuous infusion of the three dogs approximated the order described for single injections. In two animals isoproterenol was approximately seven times as effective as levarterenol which in turn had one and a half times the effectiveness of l-epinephrine. In

the third dog sixteen times as much levarterenol as isoproterenol was required to produce the same response. The maximum response to levarterenol in this animal only slightly exceeded l-epinephrine response.

D. *Ventricular responses in anesthetized animals*

Procedure. Four dogs were anesthetized with about 19 milligrams per kilogram of intravenous pentobarbital. Small supplemental amounts were necessary about every sixty minutes in order to maintain light anesthesia. No airway was inserted. Respiratory rate remained greater than ten per minute in every instance. Depth of respiration did not appear to be depressed. Two micrograms per kilogram of isoproterenol hydrochloride (equivalent to 0.008 micromols per kilogram), l-epinephrine base (0.011 micromols per kilogram), and levarterenol base (0.012 micromols per kilogram) were each rapidly given intravenously four to six times on a single day. Fifteen minutes elapsed between drug injections and administration of pentobarbital.

Results. Levarterenol proved a superior ventricular chronotropic agent to l-epinephrine as judged by comparison both of maximum responses and of change in rate. The difference in maximum response, shown in Table II, was significant ($p < 0.02$) by the method of paired group comparisons in three out of four animals. Maximum responses to isoproterenol and levarterenol were

TABLE II

Maximum Ventricular Responses to Isoproterenol, l-epinephrine,* and Levarterenol* in Anesthetized Animals*

Dog	1	5672	5727	5757
I max	73.4	44.4	84.3	63.6
S.D.	3.2	3.9	1.2	1.7
(n)	(6)	(6)	(5)	(5)
L max	72.7	42.6	80.6	66.8
S.D.	4.1	2.8	10.5	3.4
(n)	(6)	(5)	(4)	(5)
E max	55.7	35.0	63.1	64.2
S.D.	3.3	3.0	6.1	2.1
(n)	(6)	(5)	(5)	(5)
L max-E max	17.0	7.6	17.5	2.6
p	<0.001	<0.01	<0.02	$0.1 < p < 0.2$
Control Mean	39.0	21.2	31.1	36.0
Range	27.0–42.5	19.2–24.0	23.0–34.9	26.2–38.7

* 2 micrograms per kilogram body weight.

not significantly different. Ectopic beats frequently appeared after both l-epinephrine and levarterenol, less frequently after isoproterenol.

E. *Comparison of atrial and ventricular chronotropic responses*

Procedure. Three dogs were anesthetized with approximately 25 milligrams per kilogram of pentobarbital and respiration was maintained artificially with oxygen. One milligram per kilogram of atropine was given intravenously at least fifteen minutes before the first sympathomimetic amine injection. Supplemental amounts of atropine, approximately 0.25 milligrams per kilogram, were given every sixty to ninety minutes. In two animals arterial blood pressure was determined by use of a Lilly manometer. Complete atropinization was repeatedly established in two animals by the lack of femoral artery blood pressure response to 10 micrograms per kilogram of freshly diluted acetylcholine chloride. The adequacy of atropine dosage was also periodically established by observing the similarity of chronotropic responses to l-epinephrine or levarterenol before and after atropine supplements. Eight to thirteen injections of isoproterenol were given in amounts ranging from 0.0001 to 0.025 micromols per kilogram. Seven to fourteen injections of l-epinephrine and levarterenol were given separately in amounts within the range of 0.0005 to 0.025 micromols per kilogram.

Results. In every animal studied levarterenol generally produced greater ventricular rate changes than equimolar amounts of l-epinephrine. In two animals the atrial responses to both agents were nearly equal while in the third levarterenol was slightly superior. Figure 4 shows the simultaneously determined ventricular and atrial responses to each drug for one animal. Isoproterenol produced the greatest acceleration of both pacemakers being approximately two to six times as potent as levarterenol in the ventricle and two to seven times as effective in the atrium. As judged from the relative positions of the straight slope in log dose graphs, such as Figure 4, atrial and ventricular pacemakers showed approximately equal sensitivity to all three drugs.

Effects of Pentobarbital

Procedure. In two trained animals the responses to 0.0045 micromols per kilogram of l-epinephrine and to the same amount of levarterenol were determined separately before and after the intravenous administration of 19 milligrams per kilogram of pentobarbital sodium. Each drug was given four times in the control period. Beginning fifteen minutes after start of anesthesia each sympathomimetic amine was again given three to four times.

In one animal a similar experiment was performed on another day but with substitution of pentobarbital vehicle for pentobarbital. Vehicle consisted of 10 per cent ethyl alcohol, 20 per cent propylene glycol and 70 per cent distilled water.

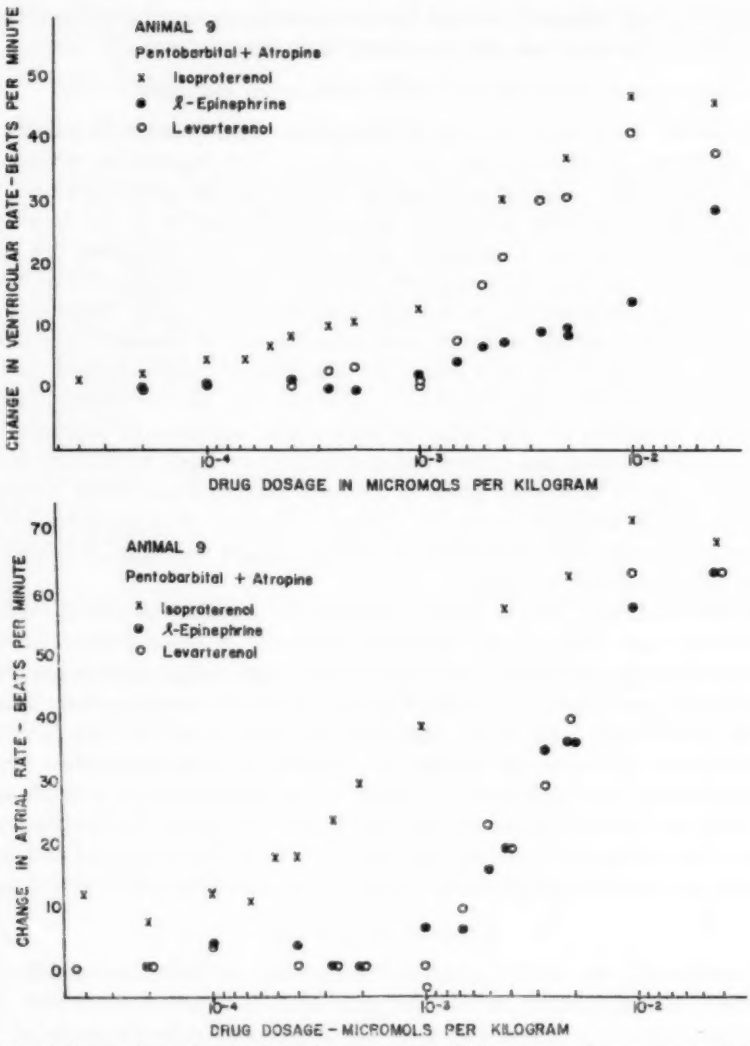

FIG. 4. Comparison of ventricular (above) and atrial (below) log dose-response relationships in an atropinized animal.

Results. Two minutes after intravenous injection of pentobarbital resting ventricular rate had increased from 35–38 beats per minute in the unanesthetized period to approximately 52 beats per minute in both of the animals studied. Subsequently resting rate decreased and within twenty-two minutes again approximated control levels. Thereafter ventricular responses to l-epi-

TABLE III

Effect of Pentobarbital on Ventricular Rate and Ventricular Response to l-epinephrine and Levarterenol**

Animal		Resting Ventricular Rate	Absolute l-Epinephrine Response	Absolute Levarterenol Response
412	Before Pentobarbital	35.5 ± 1.2 (8)	51.3 ± 1.9 (4)	56.6 ± 1.5 (4)
	After Pentobarbital	36.1 ± 0.6 (8)	55.4 ± 1.3 (4)	62.0 ± 2.5 (5)
	p value for difference	>0.10	<0.02	<0.01
549	Before Pentobarbital	37.7 ± 3.0 (10)	59.3 ± 2.0 (5)	67.2 ± 1.3 (5)
	After Pentobarbital	39.5 ± 1.2 (10)	64.7 ± 0.9 (5)	72.8 ± 1.9 (5)
	p value for difference	>0.05	<0.001	<0.001

* 0.0045 micromols per kilogram.

nephrine and levarterenol were repeatedly assayed for at least sixty-five minutes and found to be persistently greater than in the control period. These differences were highly significant, as noted in Table III.

Injection of pentobarbital vehicle was without effect in the one animal studied.

Effects of Hydrocortisone

Procedure. Three unanesthetized trained animals were employed. Resting rate and responses to l-epinephrine and levarterenol, 0.0045 micromols per kilogram, were determined at least three times before and again after the intravenous administration of 33 milligrams per kilogram of hydrocortisone sodium succinate. Fifteen minutes elapsed between hydrocortisone injection and the first succeeding sympathomimetic amine trial. All studies were complete within ninety minutes of steroid administration.

Results. Hydrocortisone had no significant effect in any animal on resting rate or response to either drug.

Effects of Acid-base Changes

Procedure. The order of experiments and methods of acid-base alteration are summarized in Table IV. The general plan of study involved repeated estimation of blood pH and ventricular chronotropic responses to l-epinephrine and/or levarterenol during control and subsequent experimental periods. Sympathomimetic amine injections of 0.003 micromols per kilogram were given in Experiments 5 and 7 while 0.0045 micromols per kilogram were given in Experiments 1–4, 6 and 8. The two figures in each of the last four columns of Table IV represent conditions before and, where possible, at the very end of the alteration indicated. Where possible all values are taken as the mean

TABLE IV

Methods and Results of Acid-base Alteration

Dog	Experiment*	Anesthesia	Method of pH Alteration	Duration of Infusion or Ventilation (minutes)	pH Change		Change in Resting Ventricular Rate		Change in l-Epinephrine† Response		Change in Levarterenol† Response	
412	1A	None	135 ml 0.6N HCl	20	7.42	6.89	41.6	26.4	55.4	29.3	55.4	34.8
	1A	None	recovery	130	6.89	7.21	26.4	38.3	29.3	53.2	34.8	59.1
	2A	None	140 ml 0.6N NaCl	21	none		41.4	45.6	52.4	53.6	59.6	61.3
	2B	None	150 ml 0.6N $NaHCO_3$	24	7.37	7.52	39.8	50.5	51.0	62.2	62.0	66.6
	2B	None	40 ml 0.9N $NaHCO_3$	7	7.52	7.59	50.5	50.0	62.2	59.0	66.6	66.9
	2B	None	125 ml 0.6N HCl	55	7.50	7.06	50.1	39.6	60.1	48.4	62.7	52.4
	3A	Pentobarbital	25% CO_2 in O_2	33	7.30	6.92		27.5		40.5		47.8
	3A	Pentobarbital	33% CO_2 in O_2	27	6.92	6.81	27.5	25.6	40.5	33.2	47.8	40.4
	3A	Pentobarbital	10% CO_2 in O_2	17	6.81	7.10	25.6	31.0	33.2	46.1	40.4	50.2
	3A	Pentobarbital	recovery	16	7.10	7.25	31.0	36.7	46.1	49.8	50.2	53.9
	3B	Pentobarbital	hyperventilation	122	7.29	7.54	34.0	42.4	53.2	61.0	57.0	67.1
	3B	Pentobarbital	recovery	21	7.54	7.22	42.4	33.6	61.0	48.0	67.1	51.3
549	4	None	140 ml 0.6N $NaHCO_3$	20	7.39	7.60	39.0	51.0			74.3	82.4
541	5A	Pentobarbital	hypoventilation on O_2	60	7.28	7.13	35.7	24.2	49.0	35.6	53.3	44.4
	5A	Pentobarbital	10% CO_2 in O_2	20	7.13	7.06	24.2	19.8	35.6	29.3	44.4	37.3
	5A	Pentobarbital	recovery	60	7.06	7.25	19.8	29.1	29.3	36.8	37.3	41.5
	5B	Pentobarbital	hyperventilation on O_2	74	7.29	7.51	31.2	30.1	36.2	43.2		52.0
	5B	Pentobarbital	recovery	31	7.51	7.25	30.1	30.3	43.2	35.9	52.0	43.0
	6A	Pentobarbital + succinylcholine	17.5% CO_2 in O_2	38	7.32	6.91	28.4	14.5			42.5	22.8
	6A	Pentobarbital + succinylcholine	12.5% CO_2 in O_2	19	6.91	6.95	14.5	16.0			22.8	23.5
	6A	Pentobarbital + succinylcholine	7.5% CO_2 in O_2	18	6.95	7.06	16.0	16.5			23.5	30.0
	6A	Pentobarbital + succinylcholine	recovery	20	7.06	7.32	16.5	22.7			30.0	35.9

	6B	Pentobarbital + succinylcholine	130 ml 0.6N HCl	53	7.42	6.94	25.3	20.7			41.0	23.6
JM 100	7A	Pentobarbital	20% CO_2 in O_2	14	7.30	7.00	24.3	10.9	33.1	22.8	34.2	24.1
	7A	Pentobarbital	10% CO_2 in O_2	4	7.00	7.12	10.9	18.5	22.8	28.8		
	7A	Pentobarbital	recovery	15	7.12	7.27	18.5	23.0	28.8	28.5		32.3
	7A	Pentobarbital	hyperventilation	12	7.27	7.47	23.0	30.1	28.5	32.2		
	7B	Pentobarbital	hyperventilation	28	7.35	7.66	23.0	32.1	32.7	41.2	33.3	43.3
	7B	Pentobarbital	recovery	48	7.66	7.30	32.1	26.0	41.2	28.4	43.3	29.4
	7C	Pentobarbital	60 ml 0.6N HCl	13	7.22	6.43	24.0	33.1	32.0	38.6	33.6	
	7C	Pentobarbital	recovery	37	6.42	6.85	33.1	16.5	38.6	22.3		25.3
	7C	Pentobarbital	80 ml 0.9 $NaHCO_3$	27	6.85	7.40	16.5	17.0	22.3	25.0	25.3	27.0
	7C	Pentobarbital	hyperventilation	21	7.42	7.46	17.0	28.8	25.0	34.6	27.0	34.4
	8A	Pentobarbital + succinylcholine	17.5% CO_2 in O_2	14	7.46	6.99	29.3	8.1			50.1	14.5
	8A	Pentobarbital + succinylcholine	12.5% CO_2 in O_2	22	6.99	7.02	8.1	15.0			14.5	21.6
	8A	Pentobarbital + succinylcholine	7.5% CO_2 in O_2	15	7.02	7.10	15.0	19.6			21.6	28.0
	8A	Pentobarbital + succinylcholine	recovery	10	7.10	7.41	19.6	33.6			28.0	40.1
	8B	Pentobarbital + succinylcholine	45 ml 0.6N HCl	13	7.45	6.76	31.4	17.0			46.5	
	8B	Pentobarbital + succinylcholine	recovery	22	6.76	6.95	17.0	22.0				26.7

* Experiments with same number performed on the same day in the order indicated by letter.
† 0.0045 micromols per kilogram except in experiments 5 and 7 where 0.003 micromols per kilogram were used.

of several determinations. Changes in rate and responsiveness approximate, within a few minutes, the changes noted for pH. All pH values are for caval blood except experiments six and eight in which arterial blood was used. At least five minutes elapsed between drug injections. No more than ten per cent of any animal's blood volume, assumed to be eight per cent of body weight, was withdrawn on any single day. With one exception at least one month elapsed between experiments on any single animal. During the interim animals were fed an iron-rich diet.

Experiments 1, 2 an 4 were performed on trained unanesthetized animals. The remainder of studies were obtained on animals anesthetized with pentobarbital in which a cuffed endotracheal tube had been inserted. During all control periods respiration was maintained artificially. The initial dose of pentobarbital was 23–26 milligrams per kilogram. Supplemental amounts were required at one to three hour intervals in order to maintain light anesthesia and freedom from shivering. Transient increase in ventricular rate usually followed pentobarbital supplements. At least twenty minutes elapsed between such supplements and the next sympathomimetic amine injection. No single experiment, indicated in Table V by a number and upper case capital letter, was interrupted by pentobarbital administration.

A. *Effects of metabolic acidosis* (Experiments 1A and 7C, Table IV)

Infusion of hydrochloric acid in Experiment 1A slowed both atrial and ventricular resting rates and decreased ventricular chronotropic responses to l-epinephrine and levarterenol. Such depressant effects were most evident at

TABLE V

Comparison of the Effects of CO_2 and HCl on Ventricular Rate and Response to Levarterenol

Experiment		Resting Atrial Rate	Resting Ventricular Rate	Maximum Ventricular Response to Levarterenol*	Arterial pH	Arterial Plasma K
6A	Control	113	28.4	42.5	7.32	3.8
	17.5% CO_2	69	14.5	22.8	6.93	4.4
6B	Control	93	25.3	41.0	7.41	3.5
	HCl	96†	20.0†	23.6†	6.94†	4.5†
8A	Control	115	29.3	50.1	7.46	4.4
	17.5% CO_2	47†	8.1‡	14.5‡	6.99‡	4.6‡
8B	Control	97	31.4	46.5	7.45	4.5†
	HCl	69	22.0	26.7	6.92	3.5

* 0.0045 micromols per kilogram. Each figure, except where noted, is the mean of three determinations.

† Mean of two determinations.

‡ One determination.

a caval pH of less than 7.10 (Fig. 5, lower left section). In Experiment 7C pH reduction was initially profound (6.43) but paradoxically accompanied by an increase in ventricular rate and a lesser increase in drug sensitivity. Atrial rate, however, fell with pH decline. With recovery, as the pH rose to approximately 6.85, all parameters became depressed and remained so even after caval pH was in the normal range. Following the infusion of sodium bicarbonate and further pH increase control values reappeared. Values greater than normal developed with hyperventilation although there was little further change in pH. No ectopic beats were observed.

B. *Effects of metabolic alkalosis* (Experiments 2B and 4, Table IV)

Alkalosis was accompanied by increased ventricular resting rate and increased responsiveness to l-epinephrine and levarterenol. Measurement of drug responsiveness was repeatedly made during both rise and fall of pH in

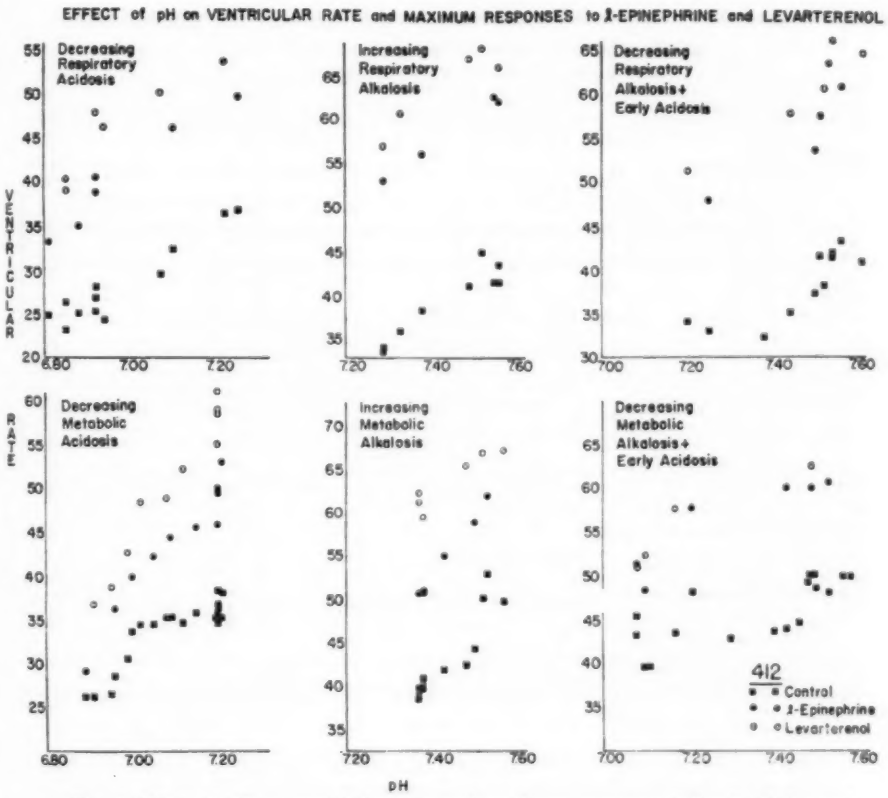

FIG. 5. Effect of pH on ventricular rate and maximum responses to l-epinephrine and levarterenol in one animal. Drug dosage is 0.0045 micromols per kilogram.

Experiment 2B but limited to the phase of decreasing alkalosis in Experiment 4. In Experiment 2B the difference between the mean response to l-epinephrine before and after alkalinization was highly significant ($p < 0.001$) as was the difference between mean levarterenol responses ($p < 0.002$). These responses are shown graphically in lower middle panel of Figure 5. The response to l-epinephrine during the alkalotic period of Experiment 4 could not be accurately gauged due to interfering arrhythmias. Such arrhythmias were frequently prefibrillatory in nature, and on the second drug trial, while the pH was falling, gave way to fatal ventricular fibrillation as shown in Figure 6. Levarterenol did not produce ectopic beats in this animal (549); however, in another animal (412), prefibrillatory activity repeatedly developed after both l-epinephrine and levarterenol. In the latter instance these effects were confined to the period of decreasing alkalosis. Ectopic beats very rarely followed drug injections at normal pH values in any animal and then were always bigeminal

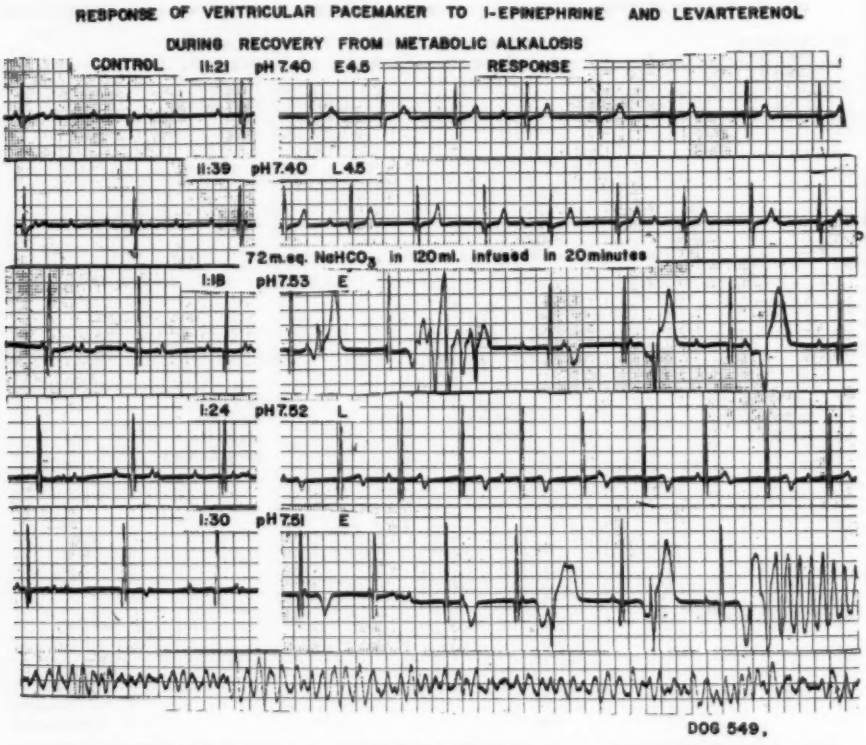
RESPONSE OF VENTRICULAR PACEMAKER TO I-EPINEPHRINE AND LEVARTERENOL
DURING RECOVERY FROM METABOLIC ALKALOSIS
CONTROL 11:21 pH 7.40 E 4.5
RESPONSE
11:39 pH 7.40 L 4.5
72 m.eq. NaHCO3 in 120 ml. infused in 20 minutes
1:18 pH 7.53 E
1:24 pH 7.52 L
1:30 pH 7.51 E
DOG 549.

FIG. 6. Effect of recovery from metabolic alkalosis on responses to l-epinephrine and levarterenol. Values to far left indicate time of day. Drug dosage is 0.0045 micromols per kilogram.

in form. Although arrhythmias often precluded exact estimation of drug responses it can be seen in the lower right panel of Figure 5 that the return of resting rate and responses to control values lagged far behind the fall in pH. Indeed control resting and response values did not reach normalcy until pH had fallen to less than 7.20 with the aid of 0.6N hydrochloric acid.

The infusion of hypertonic sodium chloride in Experiment 2A had scant effect on resting rate. The difference between mean l-epinephrine response before and after sodium chloride infusion is not significant ($p < 0.25$) nor is the difference in levarterenol response ($p < 0.25$).

Both experiments of this section were performed on unanesthetized dogs and the effects on atrial rate were too variable to allow any conclusion.

C. *Effects of respiratory acidosis* (Experiments 3A, 5A and 7A, Table IV)

Procedure. Following a control period animals were allowed to spontaneously breathe mixtures of gradually increasing carbon dioxide content in oxygen. The percentage of each gas delivered from tanks of 100 per cent oxygen and 100 per cent carbon dioxide was monitored by gas flow-meters. The resultant mixtures were delivered to a small anesthesia bag and in turn to a non-rebreathing valve attached to the endotracheal tube. A maximum of 20 to 30 per cent carbon dioxide was continued long enough (Table IV) to permit several injections of sympathomimetic amines and was thereafter gradually decreased to a second control level. Subsequently, on the same day, one animal was given an infusion of 0.6N HCl followed by 0.6N $NaHCO_3$ and hyperventilation (Experiment 7C, Table V).

Results. Hypoventilation (Experiment 5A) and administration of carbon dioxide (Experiments 3A, 5A and 7A) invariably depressed caval pH, resting atrial and ventricular rates, and responsiveness to sympathomimetic amines as illustrated in Figures 5 and 7. In all experiments the slowing of atrial and ventricular rates were more or less proportional. In one instance (Experiment 7A) drug effect was assayed two minutes after beginning carbon dioxide inhalation. At that time pH had fallen from 7.30 to 7.18, resting ventricular rate from 24.3 to 11.1, atrial rate from 103 to 61, while response to levarterenol was unchanged (Fig. 7). Six minutes later pH had further decreased to 7.07, resting rates were essentially unchanged and l-epinephrine response depressed. Reduction of resting rates and responses to both drugs thereafter persisted until acidosis was relieved. In Experiment 5A ventricular rates and drug responses were studied during both induction and recovery from acidosis. The rate of return to control values again lagged behind pH changes. A similar withdrawal lag phenomenon has been described during recovery from metabolic alkalosis in 2B and during recovery from metabolic acidosis in 7C.

Arrhythmias did not develop in the experiments of this section.

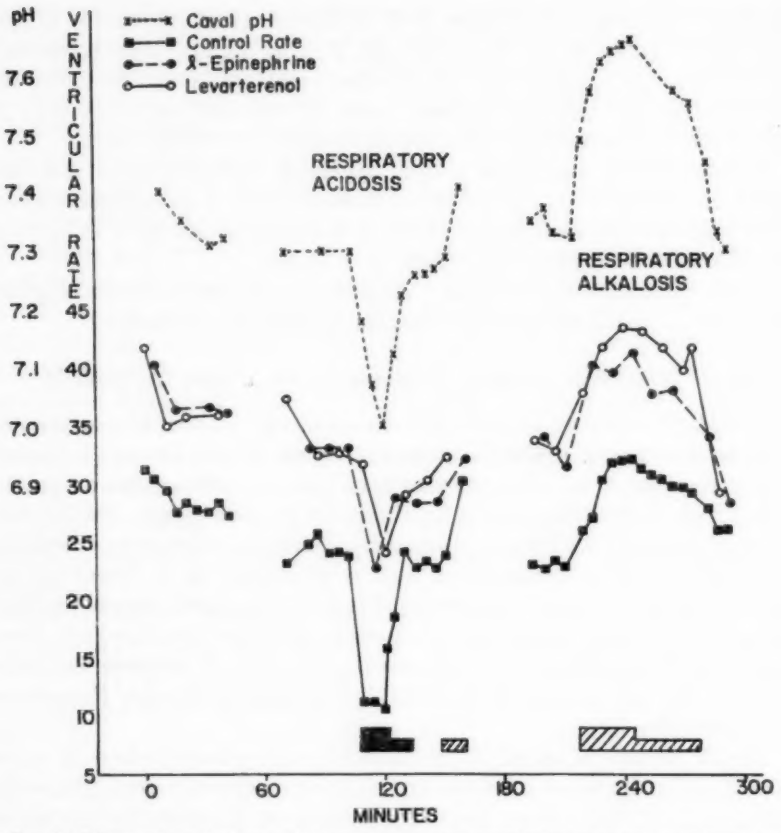

FIG. 7. Effects of respiratory acid-base changes on ventricular rate and maximum drug responses in one animal. Drug dosage is 0.003 micromols per kilogram. Values to the far left indicate changes during artificial respiration. Black bars at bottom indicate 20 and 10 per cent carbon dioxide inhalation. Shaded boxes indicate hyperventilation.

D. *Effects of respiratory alkalosis* (Experiments 3B, 5B and 7B, Table IV)

Procedure. Following recovery from respiratory acidosis animals were respired artificially. After a second control period respiratory rates and inspiratory pressures were gradually increased sufficiently to elevate caval pH to approximately 7.51 to 7.60. Hyperventilation was maintained for the periods indicated in Table V. Respiratory rates and inspiratory pressures were then gradually decreased to the control level.

Results. Hyperventilation when productive of pH values somewhat greater than 7.50 was accompanied by elevated resting ventricular rate and increased pacemaker responsiveness to both sympathomimetic amines in Experiments

3B and 7B. That these changes are significant is evident from inspection of Figures 5 and 7. The responses to respiratory and metabolic alkalosis appear to be grossly similar in Figure 5 (upper and lower middle panels) despite anesthesia in one experiment and not in the other. During recovery from respiratory alkalosis there was in this case no lag in the return to normal of resting rates and responses. Hydrogen ion concentration was less affected in Experiment 5B and resting rate was not changed although responsiveness was increased.

Ectopic beats and prefibrillatory activity during the period of decreasing alkalosis appeared after l-epinephrine injection in Experiment 3B and after both l-epinephrine and levarterenol in Experiment 5B. The only instance of arrhythmias developing during increasing alkalosis of any type occurred in Experiment 5B after levarterenol. Experiment 7B was singularly free of arrhythmias at all times.

Atrial responses were estimated in Experiment 7B and rather exactly paralleled changes in ventricular rate. In terms of absolute values in beats per minute atrial responses exceeded ventricular responses by a factor of approximately two.

E. *Comparison of the effects of metabolic and respiratory acidosis* (Experiments 6 and 8, Tables IV and V)

Respiratory and metabolic acidosis in the preceding experiments were usually produced on different days. Consequently accurate comparison of the relative effects of the two types of acidosis was not possible. Such comparisons were therefore made in Experiments 6 and 8.

Procedure. Two animals were studied and were artificially respired throughout the experiments. During both control and experimental periods intravenous succinylcholine dichloride was delivered by a constant infusion pump at a rate, 2 to 3 milligrams per kilogram per hour, sufficient to prevent spontaneous respiration and shivering. Volume infused was 6 to 12 milliliters per hour. pH was measured on whole blood samples obtained from inlying femoral artery polyethylene tubing. Hematocrits were identical at the beginning and end of experimental days. Animals were kept covered and were warmed by an electric light. Rectal temperature fell about 2 degrees centigrade during the course of an experimental day.

Arterial pH, arterial plasma potassium, and reponses to levarterenol were each measured three times during a control period while the animal was breathing 100 per cent oxygen. A minimum of seven minutes elapsed between any levarterenol injection and potassium sampling. Seventeen and one-half per cent CO_2 in oxygen was next given from a specially prepared tank and periodic measurements made of pH, potassium and resting ventricular rate. Twelve to fifteen minutes were allowed for equilibrium on this gas mixture and levarterenol responses again determined at seven minute intervals. Following one to

three such determinations and additional pH and potassium measurements the inspired CO_2 concentration was successively reduced to 12.5, 7.5 and 0 per cent as indicated in Table IV. Measurements of potassium, pH, resting ventricular rate and drug response were made during each reduction stage.

Thereafter room air was substituted for oxygen and the animals allowed to rest for one hour. At the end of this time oxygen was reinstituted and, after fifteen minutes, the control measurements repeated. Hydrochloric acid (0.6N) was then given intracavally, arterial pH and potassium determined serially, and an attempt made to match the pH depression which had been obtained with carbon dioxide inhalation. Total amounts of acid required together with time of infusion are indicated in Table IV. The first levarterenol responses of the experimental period were determined upon reaching the desired pH. This occurred forty-four (Experiment 6B) and eighteen (Experiment 8B) minutes after beginning acid infusion.

Total duration of experiment was five hours for animal 541 and three and one half hours for animal JM100. Both dogs were active twenty-four hours later.

Results. The results of both experiments are shown in Tables IV and V. In Experiment 6 depression of pH to approximately 6.94 by either means produced similar decreases in levarterenol sensitivity; however, acidosis induced by carbon dioxide was a much more powerful depressant of resting atrial and ventricular rates than the same degree of acidosis induced by hydrochloric acid. In Experiment 8 resting rates and response to levarterenol were far more depressed by carbon dioxide than by a more severe metabolic acidosis (Table V). In Experiment 8A reduction of inspired carbion dioxide concentration from 17.5 per cent to 12.5 per cent produced scant change in arterial pH but great change in drug sensitivity and atrial and ventricular resting rate (Table IV). Little change in these parameters occurred during a similar period in Experiment 6A.

Changes in arterial plasma potassium levels bore no consistent relationship to resting rates, response to levarterenol or to pH. During the first ten minutes of carbon dioxide administration potassium concentration fell from 3.5 to 2.9 milliequivalents per liter in Experiment 6A and from 4.3 to 4.1 milliequivalents per liter in Experiment 8A. Thereafter plasma potassium levels rose steadily in both experiments despite increasing pH, ventricular rate, and drug sensitivity.

A maximum potassium concentration of 4.9 milliequivalents per liter was reached in Experiment 6A, twenty minutes after stopping all carbon dioxide at a time when pH was 7.32. In Experiment 8A a maximum concentration of 5.0 milliequivalents per liter appeared during inhalation of 7.5 per cent carbon dioxide. Metabolic acidosis in Experiment 6B was associated with slowly rising potassium concentrations; during a sixty minute period of pH fall from 7.45

to 6.80 plasma potassium increased from 3.5 to 5.0 milliequivalents per liter. In Experiment 8B pH initially fell from 7.41 to 6.76 and potassium rose from 5.0 to 5.8 milliequivalents per liter. Subsequently pH increased to 6.95 and potassium fell to 3.5 milliequivalents per liter.

DISCUSSION

Relation Between Resting Rate and Response

In humans with complete heart block Gilchrist (17) has observed an inverse relationship between resting ventricular rate and the magnitude of *change* in rate produced by adrenaline. Slow resting rates were accompanied by considerable drug induced acceleration, fast rates by slight response. Such a relationship was also found in the dog. Dependence of response upon resting state is not limited to pacemakers but applies to a variety of other measurements including blood pressure and nictitating membrane contraction (18, 19).

For our purposes the most important aspect of these relationships was the comparative fixity on any single day of *maximum* or *absolute* ventricular response to l-epinephrine or levarterenol. This feature permitted the study of factors influencing these drug responses and factors influencing resting rate to proceed independently and simultaneously.

Comparison of Sympathomimetic Amine Responses

As a ventricular chronotropic agent levarterenol was usually significantly superior to equimolar amounts of l-epinephrine. Isoproterenol was approximately ten times more effective than l-epinephrine. The only previous attempt to gauge the pharmacologic reactivity of ventricular pacemakers in intact laboratory animals was made by Roberts and colleagues (20). In their experiments, performed on anesthetized animals, sympathomimetic amines were assayed for their capacity to produce ventricular escape in the presence of sinus bradycardia induced by vagal stimulation. Threshold comparisons in dogs and cats indicated isoproterenol was approximately ten times more potent than either l-epinephrine or levarterenol. The threshold dose, approximately 10 micrograms of base per kilogram body weight, for the latter two agents in dogs was twice the largest amount commonly employed in the present study and many times the amount necessary for appreciable ventricular acceleration. In addition to these differences there is a further discrepancy between the results of the two investigations. The most rapid responses observed in our experiments on unanesthetized animals were usually associated with QRS complexes of less than 0.07 seconds duration. This duration is within the limits described for dogs with normal sinus rhythm (21) and therefore indicates a pacemaker situated in the bundle of His. In contrast the ventricular responses illustrated by Roberts attained rates of 100–150 beats per minute and had wide QRS complexes suggesting an origin distal to bifurcation of the A-V

bundle. As an explanation for such wide QRS responses it seems possible that the flow of impulses from the partially inhibited sino-atrial pacemaker remained sufficiently rapid to prevent the development of bundle pacemakers but at the same time sufficiently slow to allow the interposition of ectopic ventricular rhythms. In other words, a variant of ventricular tachycardia was produced with equal success by l-epinephrine and levarterenol while isoproterenol was ten times as potent in this respect. Some confirmation of this explanation comes from our experience with anesthetized "heart block" animals in which repeated 2 microgram per kilogram injections of l-epinephrine, levarterenol and isoproterenol were often followed by ventricular tachycardia. The QRS complexes of such tachycardias were usually considerably wider than observed in the resting state.

Nathanson and Miller (22) have described the ventricular chronotropic effects of intravenously administered isoproterenol, l-epinephrine, and levarterenol in eight patients with complete heart block. If these patients are assumed to have averaged seventy kilograms in weight and the drug doses employed are expressed in micromols per kilogram it is possible to compare ventricular pacemaker sensitivity in dog and man. Such gross comparison indicates that sensitivity to isoproterenol is the same in the two species. In contrast humans with complete heart block are approximately thirty times more sensitive than dogs to l-epinephrine. Levarterenol, superior to l-epinephrine in dogs, often produced arrhythmias in humans and never yielded rate increases greater than 11 beats per minute; consequently quantitative comparison of this agent is not possible. Doubtless a quantitative difference exists between the two species for ventricular pacemaker sensitivity to levarterenol just as it does for blood pressure response. As measured by the amount of drug necessary to produce equivalent systolic pressure elevation man (23) is three to four times more sensitive to levarterenol than are dogs (24). Precedent is also available for qualitative, species determined, differences in drug responses (25). However, several factors suggest the need for some other explanation for qualitative differences in ventricular sensitivity to levarterenol and l-epinephrine between dog and man. The first of these factors is the qualitative similarity of atrial responses in the two species before atropinization. Following atropinization atrial rate is accelerated by levarterenol in both dogs (26) and humans (23). The parallel sensitivities shown by atrial and ventricular pacemakers in the dog, Figure 4, raises the problem as to why these pacemakers should not behave similarly in humans. The interplay between vagal and sympathetic forces upon the atrioventricular node provides a possible explanation for the differences in question. The pacemaker of the dog with complete heart block cannot lie higher than the bundle of His. Vagal stimulation has no effect on such pacemakers (10). These pacemakers are therefore only subject to the acceleratory action of sympathomimetic amines. If a conduction block

in man is confined to the upper or middle node then the pacemaker may lie in the middle or lower node and there be subject to a balance of sympathetic and vagal effects. Levarterenol, through its effect on blood pressure, is a powerful stimulant of baroceptor vagal reflexes and therefore a poor nodal excitant. The escape of still lower effective pacemakers is not likely to occur because, as Roberts' experience indicates, comparatively large amounts of drug are necessary for their excitation. Ectopic beats may still develop, particularly when basic rate remains slow and allows their interposition. l-Epinephrine is a lesser and isoproterenol a poor baroceptor stimulant. Consequently the direct acceleratory action of these drugs on the atrioventricular node would predominate.

The correctness of the explanation just advanced could be established in patients with complete heart block by testing the ventricular chronotropic responses to small amounts of l-epinephrine and levarterenol before and after atropinization. If the explanation is correct then levarterenol response should improve after vagal blockade. Some reservation will still be necessary concerning interpretation as to the site of conduction defect, since it is possible that vagal influence in man penetrates to the His bundle. In the rabbit Hoffman (27) has recently demonstrated that A-V node pacemakers are sensitive and bundle pacemakers insensitive to acetylcholine. Doubtless the level of vagal influence differs slightly between species and perhaps between individuals within species. However, if vagal sensitivity in man extends no further than the A-V node, then complete heart block could be regarded, at least in those cases poorly responsive to levarterenol, as a disease confined to the upper portions of the A-V node. Stokes-Adams disease might represent an extension of this process to the lower node and bundle.

The Effect of Pentobarbital

Transient increase in ventricular rate followed initial and supplemental pentobarbital injections. Similar transient effects upon atrial rate have recently been reported (28). It is possible that such changes reflect hypersensitivity to endogenous sympathetic activity. This view is supported by the subsequent demonstration of increased ventricular chronotropic responses to l-epinephrine and levarterenol. Hypersensitivity alone is not responsible for resting rate alteration since heightened responsiveness persists long after resting rate has returned to normal. Brief sympathetic or neurohormone discharge is one alternative mechanism and altered pacemaker metabolism another.

Lockett (29) found in the atropinized dog that the atrial pacemaker responsiveness to epinephrine is augmented by pentobarbital while responsiveness to arterenol is depressed. To account for these findings it was suggested that methylation is a prerequisite to arterenol activity and that pentobarbital depresses enzymatic methylation. In our experience pentobarbital increases

ventricular pacemaker sensitivity to both amines. It seems unlikely that methylation would be essential for the action of arterenol on one pacemaker but not on the other.

The Effect of Hydrocortisone

Lown and co-workers (4) observed that the P–R interval is prolonged in Addison's disease and short in Cushing's syndrome when compared to a normal population. The use of adrenocortical steroids in complete heart block was consequently proposed with the hope of establishing A-V conduction. The mechanism by which conduction might be enhanced was thought to involve adrenal steroid—neurohormone interaction. Our study sought to determine if this interaction extended to the ventricular pacemaker. We could demonstrate no such interaction. Similar findings have also been observed in isolated perfused guinea pig heart (30).

The Effect of Acid-Base Changes

The mechanism by which hypertonic lactate affects ventricular rhythmicity has not been clarified. Alkalinization, whether produced by decrease in CO_2 concentration (31, 32) or increase in bicarbonate (32) content, had no effect on the heart rate of perfused rabbit auricles. However, Andrus (31) found that there was a greater chronotropic response to adrenaline at pH 8 than at pH 7. The interpretation from these observations that alkali enhances atrial response to adrenaline should be made with care since drug response at a normal pH was not determined. The disparity between resting rate and adrenaline sensitivity in isolated atria suggests that increased resting ventricular rates in alkalotic "heart block dogs" may be produced by hypersensitivity to endogenous sympathetic activity or circulating neurohormones. Increased sympathetic discharge or neurohormone secretion cannot be excluded as an alternative explanation. There is, however, indirect evidence for diminished vasoconstriction in arm muscle during hyperventilation in man indicating that sympathetic activity may actually be reduced in alkalosis (33).

The arrhythmias which accompany alkalosis in the dog with complete heart block are largely confined to the recovery period and then only appear following injection of sympathomimetic amines. Arrhythmias developing in humans with heart block treated with intravenous hypertonic sodium lactate rarely persist after stopping the infusion (7). However, one individual (patient 4, reference 7) spontaneously exhibited bursts of bizarre ectopic beats accompanied by chest pain at a time following the infusion of 0.5M sodium lactate when venous pH was decreasing. Such beats, not present in the control period, closely resembled the prefibrillatory activity appearing in dogs. The phenomenon of post-alkalotic arrhythmias may not be limited to dogs. Possibly its occurrence in humans not concurrently receiving sympathomimetic amines is

related to transient increase in endogenous sympathetic activity and neurohormone discharge. The mechanism which ultimately predisposes to these disturbances in rhythm is unknown. Differential shifts in certain ions, particularly potassium and calcium, may be involved. Increase of the calcium-potassium ratio in the perfusate of rabbit heart is known to initiate ventricular fibrillation (34). During the post-hyperventilatory period serum ultrafiltrable calcium is elevated to greater than control levels (35). At the same time the depressed serum potassium concentration of alkalosis persists in the recovery period (36). These changes, though minor, may predispose to the excitation of ventricular fibrillation.

In vivo acidosis depressed ventricular and atrial rates and diminished responsiveness to l-epinephrine and levarterenol. *In vitro* studies of isolated perfused rabbit atria also reveal a diminished rate with acidosis (31, 32). It is therefore unlikely that diminished resting rates in the acidotic dog result solely from decreased sensitivity to endogenous sympathetic or neurohormone activity. This view is supported by the disparity between resting rate and drug densitivity in Experiment 7A (Fig. 7). In this case resting atrial and ventricular rates fell precipitously with decreasing pH at a time when pacemaker sensitivity to levarterenol remained in the control range. Decreased sympathetic discharge and neurohormonal secretion do not seem likely causes of resting rate bradycardia since acidosis is usually regarded as a stimulus to such activity (19, 37). Indeed the initial paradoxical increase of ventricular rate with marked acidosis found in Experiment 7C is probably attributable to sympathetic discharge sufficient to overwhelm depressant effects of increased hydrogen ion concentration. It is curious that in this latter experiment atrial rate decreased concurrently with ventricular acceleration. The explanation for such atrial slowing may lie in increased sensitivity to vagal tone which has been described in acidosis (38).

As indicated in Table V carbon dioxide inhalation produced a much greater slowing of rate than did an equivalent pH depression following hydrochloric acid infusion. These findings are perhaps analogous to the observation that arterial blood when acidified with carbon dioxide had a greater effect upon respiration than when acidified by hydrochloric acid (39). Vaughan Williams (32) has demonstrated that alteration of carbon dioxide concentration alone has no consistent effect on the heart rate of perfused rabbit atria. Cellular membranes are believed to be comparatively porous to carbon dioxide but almost impermeable to hydrogen ion (40). If this is true the discrepancy between the effects of metabolic and respiratory acidosis could be explained by assuming that intracellular rather than extracellular acidosis is a major determinant of resting rate depression.

The relation of extracellular potassium to the effects associated with depression is uncertain. Hyperkalemia is often associated with sinoatrial bradycardia

(41) and serum potassium concentration is usually elevated in acute acidosis (41, 42, 43). However, in our experience extracellular potassium concentration in acute acidosis is at best only a minor determinant of ventricular rate. For example, potassium may increase by fifty per cent (Experiment 6A) while pH and rate remain comparatively steady. On the other hand, persistent potassium alteration during recovery periods may account for delays in the return of resting rates to normal.

The reasons for decreased response to levarterenol in acidosis are even less clear. Table V indicates that in one animal decreased sensitivity correlated well with lowered blood pH irrespective of the means by which this was produced. Responses in the other animal were more depressed by carbon dioxide than by hydrochloric acid. This discrepancy may be due to bradycardia and associated poverty of venous return sufficient to permit partial destruction of levarterenol before delivery to the heart. A similar criticism may indeed apply to the whole concept of the measurement of response without regard to resting rate. Undoubtedly there is for each animal some minimum value for resting rate below which response is related to preceding control levels. However, the data for Experiments 6A and 6B in Table V indicate that resting rates of an order of 14 to 20 beats per minute are associated with the same absolute drug response. Similar results were obtained in the early phase of Experiment 7A over a resting rate range of 11 to 24 beats per minute. An alternative explanation for these findings would be that absolute response *is* related to resting rate and acidosis *increases* sensitivity to sympathomimetic amines. This explanation seems unlikely since in other experiments proportionate or disproportionate changes in resting rate and response to drugs would indicate that acidosis has no effect on sensitivity or decreases it. We therefore feel justified in the use of absolute response as an index of sensitivity to sympamimetic amines. It must be recognized, however, that absolute responses occurring in the presence of resting rates less than 15 beats per minute may not be entirely a function of drug sensitivity.

Our observations indicate that acidosis, particularly of respiratory origin, should be promptly recognized and treated in patients with complete heart block. When no acid-base derangement is initially present alkalinization may be quite hazardous. The sensitivity of ventricular pacemakers to acidosis probably varies in man just as it does in dogs. It is conceivable that a mild degree of pH depression is responsible in some persons for refractoriness to sympathomimetic amines. In this connection Lillehei (44) has observed restoration of isoproterenol effectiveness following administration of molar sodium lactate in patients who had developed complete heart block during open heart surgery. Levine (45) has advocated the use of ammonium chloride in Stokes-Adams disease. This is an effective acidifying agent (41) and would seem undesirable if ventricular standstill is the mechanism of syncope. Possibly induced

acidosis has some value in the treatment of attacks which begin as ventricular fibrillation or tachycardia. Since multiple types of syncopal mechanisms may occur at different times in the same patient (46) ammonium chloride should always be employed with caution. Vandam and McLemore (47) have recently shown that there is good reason to anticipate circulatory arrest in patients with complete heart block undergoing anesthesia and operation. Respiratory acidosis was regarded as a possible causative factor and the suggestion advanced that better pulmonary ventilation and the injection of molar sodium lactate may enhance the response of the myocardium to endogenously released sympathomimetic amines.

SUMMARY

An attempt has been made to provide an experimental basis for the treatment of Stokes-Adams disease. Chronic complete heart block was surgically produced in dogs and chronotropic responses to sympathomimetic amines determined. In a limited number of animals the influence of a variety of agents upon chronotropic responses to l-epinephrine and levarterenol was studied.

The *change* produced in ventricular rate by l-epinephrine and levarterenol on any one day is inversely related to resting rate. *Absolute* or *maximum* drug response is comparatively fixed and independent of resting rate.

As a ventricular cardioacceleratory agent levarterenol was generally superior to equal amounts of l-epinephrine in both unanesthetized and anesthetized animals.

On a molar basis approximately six times as much levarterenol and twelve times as much l-epinephrine was required to produce the same ventricular chronotropic effect as isoproterenol in unanesthetized animals. Reflex vagal activity upon A-V nodal pacemakers in some humans with complete heart block may partially account for the difference between dog and man in response to levarterenol. A corollary of this argument suggests that the A-V node is a common site of the lesion of complete heart block in humans.

The relative sensitivities of atrial and ventricular pacemakers to isoproterenol, l-epinephrine and levarterenol are of the same general order.

Pentobarbital transiently increased ventricular rate and persistently enhanced responsiveness to l-epinephrine and levarterenol.

Hydrocortisone had no acute effect upon ventricular rate or response to l-epinephrine and levarterenol.

Acidosis of respiratory or metabolic origin resulted in slowing of resting ventricular rate and diminished sensitivity to l-epinephrine and levarterenol. Carbon dioxide depressed ventricular rate more than did equivalent degrees of acidosis induced by hydrochloric acid. This prompted the suggestion that intracellular rather than extracellular acidosis is a major determinant of bradycardia. Decreased sensitivity to cardioacceleratory drugs appeared to be

a function of blood pH. No consistent relationship was observed between plasma potassium and resting rate or responsiveness to drugs.

Alkalosis of respiratory or metabolic origin produced resting ventricular rate acceleration and enhanced responses to l-epinephrine and levarterenol. Ectopic beats were confined to the period of recovery from alkalosis and then only developed following injection of sympathomimetic amines. Prefibrillatory activity was common during this period.

It is concluded that acidosis should be promptly recognized and treated in the patient with complete heart block. When no initial acid-base derangement is present, alkalinization may be hazardous.

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THE SYNTHESIS OF GLUCOSE BY THE KIDNEY[1]

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INTRODUCTION

Three types of observations suggest that the kidney may play a role in the glucose economy of the body. First, it has been reported that nephrectomy or ligation of the ureters increases the glucose requirements of the hepatectomized animal (1). The effects of nephrectomy in this system have been confirmed (2, 3). Two other sets of observations have furthermore indicated that the kidney synthesizes glucose: 1) studies of renal arterio-venous glucose differences (4–7), and 2) studies of glucose synthesis by kidney slices *in vitro* (8–10). However, the physiological significance of these observations remains unclear for several reasons. All previous studies have used glucose assay methods which lack in optimal specificity and precision. Furthermore, in experiments on "intact" animals, the use of anesthesia, hepatectomy, ligation of the ureters and the presence of acute operative trauma has obscured the interpretations of the findings as regards the normal animal. To further complicate the problem, whereas it appears that ureteral ligation increases the glucose requirement of the hepatectomized animal (1), it does not appear to abolish the appearance of new glucose in the renal vein (4–7). Slice experiments have indicated that renal glucose synthesis is decreased in the adrenalectomized animal (10), but there is as yet no clear indication concerning how this may be related to other deficiencies of renal function seen after adrenalectomy. The entire problem appears to warrant further study from the physiological point of view.

As an initial approach, we have tried to establish more precisely whether or not, in the normal state, the kidney synthesizes glucose in excess of its requirements, and have developed experimental techniques which appear superior to those used in the past. These techniques permit the drawing of arterial and renal vein blood samples from the calm, unanesthetized dog. Glucose concentrations in these samples have been measured by a precise and specific procedure which will be described. As is to be shown, the observation that the kidney can deliver excess or "new" glucose into the renal vein has been confirmed in the normal animal. The possible significance of this fact is to be discussed. It is quite evident that the validity of our observations is entirely dependent on the validity of the experimental techniques. For this reason, the

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methods used are described in detail, with a description of studies done to evaluate potential sources of error.

METHODS

I. Implantation of Catheters

Normal male and female adult mongrel dogs were used. All were maintained on normal high protein kennel rations before and after the surgical procedures, except as noted. Two surgical approaches have been used for the implantation of catheters into the blood vessels. Our experience indicates that the second procedure is preferable. In both procedures, the dogs were anesthetized with sodium pentobarbital (30 mg/kg body weight) and prepared for sterile surgery at the chosen operative sites.

Method A: In the first four dogs to be reported, the left renal pedicle was approached retroperitoneally by means of a left flank incision, and the left spermatic vein visualized at its junction with the renal vein, using blunt dissection as necessary. The spermatic vein was ligated, and a #20 polyethylene catheter inserted into it proximally until the tip of the catheter projected about 1 millimeter into the renal vein. It was sutured securely in this position. The free end of the catheter was then brought out through a separate stab wound dorsal to the initial flank incision. At the same operation another catheter was inserted into the aorta through one of the carotid arteries. The free end of this catheter was also brought out through a separate stab wound on the dorsum of the neck.

Method B: In the second series of four dogs, the abdomen was entered by a long midline incision and the intestines rotated to the left by sweeping them over with the duodenal mesentery. This widely exposed the aorta, inferior vena cava, the right and left renal and adrenal veins and the spermatic or ovarian veins (see Fig. 1). A pursestring suture of 5-0 "arterial" silk was placed in the caval wall opposite the lumen of the left renal vein. A #T-7 polyvinyl catheter[3] was introduced through a small stab wound in the middle of the purse-string and then threaded into the left renal vein as far as the spermatic or ovarian vein, which was subsequently ligated and divided. A small suture of 5-0 silk was then placed in the wall of the renal vein and around the catheter about 3 or 4 mm from its end, thus holding it in position against the wall of the renal vein without occluding the vein. This suture, properly placed, is the key to an efficient and chronic preparation. The catheter was sutured securely at several points to the posterior abdominal wall up to a point where it was brought out through a separate stab wound in the right posterior flank. Another catheter was introduced similarly into the abdominal aorta and brought out caudad to the renal vein catheter in the flank. In two dogs the inferior vena cava was also catheterized, distal to insertion of the renal vein, with the tip of the catheter at the level of the caval bifurcation. No infections were subsequently seen at the wound or catheter sites.

In one animal, in which renal function was to be studied, the bladder was bisected after the method of Desautels (11). In this dog nylon cannulas were inserted into each bisected portion of the bladder and brought out in the lateral areas of each suprapubic region.

II. Maintenance of the Catheters

As soon as each catheter was in place in its vessel, heparinized saline[4] was instilled through an 18 gauge needle on a syringe. The catheter was then closed by a small clamp across the tip.

The greatest problem found in the maintenance of these catheters was that of preventing

[3] Surprenant Co., 199 Washington Street, Boston, Massachusetts, Catalogue #T-7. This size barely admits an 18 gauge needle into its lumen.

[4] 1 ml heparin/250 ml .85 per cent NaCl.

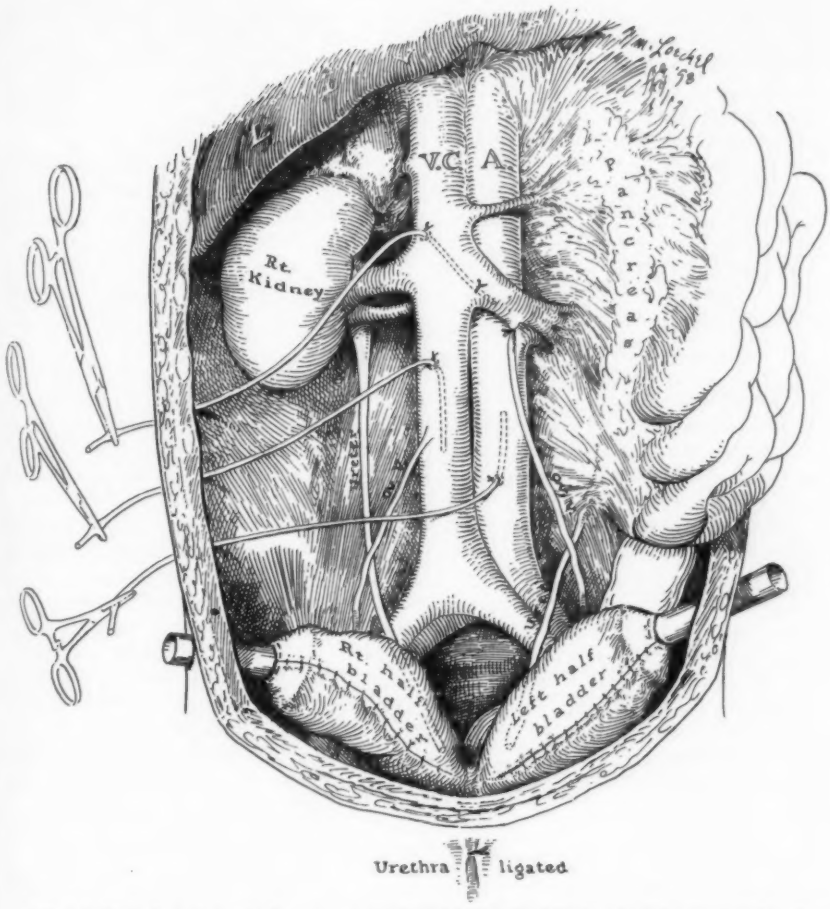

FIG. 1. Drawing of surgical exposure employed in Method B showing placement of catheters. The technique of the bladder-splitting procedure is also indicated, showing the cannulae implanted.

the dogs from chewing or pulling them out after they recovered from anesthesia. In the first four dogs studied, stockinette jackets and many yards of adhesive tape were used. During the experiments an extra person was required to restrain the dog during withdrawal of the blood samples. It was subsequently found that use of body jackets made of light canvas solved many of the problems encountered. These jackets permitted entirely normal activity of the dogs on days between experiments, and they also solved the problem of restraint during experiments. With their use, in conjunction with a restraining frame, it was found possible for a single person to carry out the experimental procedures. The jackets were tailored to fit dogs of specific weight ranges, and had four holes for the legs and a series of tags which could be tied across the back of the animal between experiments, or to the frame

for restraint during experiments. Between experiments, the heparinized catheters, clamps attached, were held against the side of the animal inside the jacket. Generally a few strips of adhesive tape were passed entirely around the abdomen before the jacket was put on, and the clamps then attached to these strips with more tape. This held the clamps in place and made it unnecessary to pull tape from the fur at the time of an experiment. On experimental days the dogs could remain in the frame for two or three hours with no distress, in an upright position. If their legs became tired they could lower themselves into the jacket and rest, which all of them did from time to time. Water was made available to them by a stand holding a water pan. As a general rule, however, they did not drink. A towel about the neck of the dog was usually clamped to the top of the frame in such a fashion that the dog could rest its head and neck. Occasionally it was necessary to have a leash on the neck, tied to the frame anteriorly, so that the head could not be turned back far enough to reach the catheters.

III. Drawing of Blood Samples

The catheters were attached to three-way stopcocks via needles. These were held tightly by clamps attached to the frame. Syringes could thus be put on and off with a single hand. When blood samples were to be drawn, each catheter was quickly cleared of anticoagulant by withdrawing two ml. of blood and fluid. The stopcocks were then closed, and dry syringes put in place. Four ml. of fresh blood were then drawn simultaneously from each catheter. These blood samples were immediately placed into bottles containing 6 mg. of dry disodium ethylenediaminetetraacetic acid with which the blood was immediately mixed by capping with parafilm and inverting. For preparation of plasma, samples from the anticoagulant bottles were quickly transferred to dry tubes in an ice bath. For whole blood studies, 2.0 ml. aliquots were quickly transferred to tubes containing .5 ml. 40 per cent trichloracetic acid, and shaken. An attempt was made in each case to centrifuge blood for plasma samples as quickly as possible, following which the plasma was stored frozen. Between sampling, heparin solution was injected into the catheters. During the average experimental run, pairs of samples were drawn roughly every fifteen minutes. Usually a total of no more than eight pairs were drawn. This resulted in a maximal blood loss to the dog of less than 70 ml. Bloody fluid removed on clearing the catheters was re-injected before instilling heparinized saline.

IV. Measurement of Glucose Concentrations in Whole Blood and Plasma

The procedure adopted was derived from a method originally described by Keston (12) and Teller (13), which depends on the reaction of glucose with glucose oxidase and thereby is virtually specific for glucose. We have carried out a series of studies with the method as used by us and with the enzyme preparations employed to make certain that in our conditions the method gives valid results. These will be presented. At the present state of our experience with the procedure, it seems advisable that these studies be repeated with each new lot of glucose oxidase used, and also if further modifications of the procedure are to be made. During the preparation of this manuscript another modification of the same principle has appeared (14) which, however, employs considerably different conditions for the assay procedure. In all of these procedures aqueous glucose solutions are incubated at room temperature in air with a buffered mixture of glucose oxidase, horseradish peroxidase and a dye, o-dianisidine (enzyme-dye reagent). This is done at glucose concentrations well below the K_m of glucose for glucose oxidase.[5] In this reaction glucose is converted to gluconic acid and hydrogen peroxide is formed in equimolar amounts. The rate of peroxide formation is

[5] The K_m has been given as 4.2×10^{-2} (15).

then measured by the oxidation of the o-dianisidine, which is catalyzed by the peroxidase. The dye is thus converted to a colored product with a broad absorption spectrum conveniently measured at 420 millimicrons. Unknown concentrations are plotted from a curve made from standards run simultaneously in each assay, after correction for reagent blank readings.

Time curve studies of the rate of color development, employing initial glucose concentrations in the ranges to be described, indicate that the reaction rate proceeds according to a "die-away" curve with a half-time of about 10 minutes. Color development is thus always directly proportional to the initial glucose concentration. Incubation times have been chosen for ease of accurate measurement and optimal optical densities for spectrophotometric assay.

a) Preparation of materials for assay

1) *Plasma.* .3 ml.[6] aliquots of plasma were mixed thoroughly with .1 ml. of 12 per cent trichloracetic acid, and the mixture centrifuged. .2 ml. of the supernatant were then mixed with an equal volume of .09 N NaOH, which made the solution slightly alkaline. This material was then stored in stoppered tubes for as long as 1 hour, until glucose assay was performed.

2) *Whole blood.* Immediately after being mixed with the anticoagulant [cf Methods III], 2.0 ml. of whole blood were transferred with a volumetric pipette into a screw capped tube[7] containing .5 ml. of 40 per cent trichloracetic acid. The cap was replaced and the tube vigorously shaken. After 24 hours in the refrigerator the tubes were centrifuged and the supernatant transferred to a clean tube, which was stoppered and stored in the refrigerator (approximately 5°C) until the time of assay. Just prior to assay, .2 ml. aliquots of this supernatant were taken to a pH somewhat above 7.0 by addition of an equal volume of .29 N NaOH. After mixing and standing about 15 minutes at room temperature, this material was recentrifuged. This latter procedure frequently resulted in the appearance of a pale, slightly pink or yellow flocculum in the bottom of the tube, the removal of which appeared to increase the reproducibility of the determinations. This last centrifugation also brought the blank readings of supernatant in water to nearly zero. No difference in blank values of arterial and renal vein blood supernatants has been seen.

3) *Enzyme-dye reagent.* This was prepared just prior to use as follows: 100 ml. .1 M potassium phosphate buffer, pH 7.1 at room temperature, was divided into two equal parts. To one aliquot was added 125 mg. glucose oxidase (Takamine) and 10 mg. horseradish peroxidase (Worthington). To the other aliquot was added 2.0 ml. of 1 per cent o-dianisidine in ethanol. After mixing separately, both aliquots of buffer and their additions were mixed together and filtered through Whatman #1 filter paper. It is to be noted that this reagent is essentially that of Teller (13), but that the buffer concentration and peroxidase concentrations have been increased, Teller having used 5 mg. peroxidase in 100 ml. .005 M buffer. .1 M buffer is strong enough to control any variations in pH in the supernatant. The reagent is stable for several hours, although blank values slowly rise on standing. The enzymes and dye are stable for months or longer when stored dry under refrigeration.

b) Assay procedure

.1 ml. aliquots of the alkalinized supernatants to be assayed were transferred in duplicate to 15 × 125 mm. tubes. In each series of determinations .1 ml. water, and 20, 40, 60 and

[6] All volumes below 1.0 ml. were pipetted with use of Lang-Levy constriction micropipettes such as have been described (16, 17).

[7] Caps sealed with Teflon (Will Corp.)

80 mg. per cent equilibrium glucose were also assayed in duplicates. Tubes and reagents were allowed to come to room temperature on the laboratory table, before the assay was started. The procedure was started by blowing 2.0 ml. aliquots of the enzyme-dye reagent into each tube successively at 15 second intervals. After a precisely timed interval, in the range of 10–12 minutes,[8] 1.0 ml. .5 N HCl was blown into each tube in the same order, also at 15 second intervals. Optical density of each tube was then measured at 420 millimicrons. The duplicate readings were then averaged and glucose concentrations of the unknowns determined from the standard plot for each series.

c) Calculations

To obtain the glucose concentration in the original blood or plasma, the concentration in the alkalinized supernatant, determined above, is multiplied by a factor of 2.1, for whole blood, and 2.5 for plasma.[9] The distortion caused by differences in water content of renal vein and arterial samples due to hemo-concentration is discussed in a later section. It can be shown that the maximal distortion is somewhat less than the difference in the water contents.

d) Specificity of the method, and alpha-beta glucose equilibrium

Glucose oxidase is highly specific for glucose, and reacts specifically with beta glucose (18). The method presented, however, measures total glucose, probably in part due to the presence of mutarotase in the commercial glucose oxidase preparations, and also in part to the fact that the supernatants used and the reaction mixture are both somewhat alkaline, which greatly increases the rate at which alpha and beta glucose reach equilibrium. Freshly

[8] Incubation time may vary from 10–12 minutes, if accurately timed. Times varying from 7–21 min. have been found suitable in checks with glucose standards, but 10.0 min. generally gives readings in the optimal range for precision. If more than 40 tubes are to be run in one assay, the incubation time must be greater than 10.0 min.

[9] In this procedure whole blood and plasma have undergone a small dilution in the preparation of the protein-free supernatants. It is therefore necessary to correct for the water contents of the original materials. The general formula is as follows:

$$G_1 = \frac{G \cdot V}{(V \cdot H) + (V_t \cdot H_t)} \times \frac{1}{2}$$

where G_1 is the glucose concentration in the alkalinized supernatant and G that of the original whole blood or plasma (mg/ml); V is the volume (ml) of whole blood or plasma used, and V_t the volume of trichloracetic acid used; H is the water content (gm/ml) of the whole blood or plasma, and H_t the water content of the trichloracetic acid used. In the assay procedure described, V is 2.00 ml. for whole blood, and .3 ml. for plasma; H is .86 gm/ml for whole blood and .94 for plasma. V_t is .500 ml. for whole blood and .100 ml. for plasma; H_t is .77 for the 40 per cent trichloracetic acid used with whole blood, and .93 for the 12 per cent trichloracetic acid used with plasma.

By rearrangement, the formula given above becomes

$$G = G_1 \frac{2\,[V \cdot H + V_t \cdot H_t]}{V}$$

By substitution of the appropriate values given above

$G = G_1 \times 2.1$, for whole blood
$G = G_1 \times 2.5$, for plasma.

prepared solutions of alpha glucose, prepared by the method of Hudson and Dale (19), have been added to plasma. Filtrates were then made, as described, and assayed against equilibrium glucose. Virtually exact recoveries were obtained. Solutions of the following carbohydrates gave no reaction with this method: Fructose-6-phosphate, glucose-6-phosphate, D levulose, D xylose, D-arabinose and D-sorbitol all at 50 mg. per cent in the added aliquot. D galactose and glycogen gave slight color development, about 3 per cent of that of glucose at the same concentrations. Keilin and Hartree (15) noted a similar rate of reaction of galactose with glucose oxidase.

It is highly unlikely that any peroxide forming substrate other than glucose could be operative in this system, and the method evidently gives true glucose measurements provided that interfering substances are removed from the supernatants assayed. Recovery experiments with the supernatants described have been highly satisfactory. Studies of diluted filtrates also indicate the absence of interfering substances.

e) Standard curve

The standard curves are nearly linear, but not exactly so. When concentrations are below 15 mg. per cent in the aliquot added to the enzyme-dye reagent, there is a slight distortion, possibly related to the presence of catalase in the glucose oxidase preparations. This could compete with the peroxidase for the H_2O_2 formed, making the standard curve slightly concave. For this reason, slight modifications of the given procedure may be desirable for assay of glucose in solutions containing less than 15 mg. per cent glucose. This presents no problem in this procedure if the glucose concentration in the fresh whole blood or plasma is above 40 to 50 mg. per cent.

f) Accuracy of the method

Estimates of the error of a single glucose concentration measurement have been made by performing two separate assays on pairs of blood filtrates and plasma. The mean difference between duplicate assays on a series of twelve plasmas was .08 mg. per cent with a standard deviation of ± 1.51 mg. per cent. For 34 duplicate assays on whole blood filtrates the mean difference was 2.1 mg. per cent, standard deviation being ± 2.3 mg. per cent. Much of this variation appears to be systematic and due to slight variations in the standards. There is much less variation between the first and second determinations of differences in concentrations (renal vein-artery). In 17 such pairs of whole blood filtrates the mean difference between the first and second measurements of pair difference in glucose concentration was .11 mg. per cent, with a standard deviation of ± 1.06 mg. per cent. A slightly different pooling of data, in which the squared deviations of each pair difference measurement from the average of the duplicates are summed, gives an estimate of the error (at the 95 per cent confidence level) of a *single pair difference measurement* as ± 1.80 mg. per cent in both plasma and whole blood.

As shown in Results, the method appears satisfactory for the purposes of the studies reported. Even though the mean arteriovenous differences found are smaller than the error for a single pair difference measurement, it can be seen that the standard errors of the means found are quite small. All analytical errors are included in the standard errors of the experimental means, together with all other possible sources of variation. It is to be expected, of course, that analytical errors will tend to cancel out in a series of measurements.

g) Comparisons with other methods

Plasma glucose concentrations determined with this method compare well with those found in Folin-Wu filtrates (20) by the method of Benedict (21), being 1 to 3 mg. per cent

lower in the glucose oxidase method. Whole blood assayed similarly before and after treatment with glucose oxidase also checked closely.[10]

h) Storage of blood samples before preparation of plasma

For preparation of plasma samples, the whole blood aliquots sometimes remained in the ice bath as long as 30 minutes before centrifugation. This raised the possibility that some of the A-V differences seen might be due to changes in the blood during the storage period. An experiment to test this possibility is described in the results (cf table V and corresponding text). No significant distortion of plasma A-V differences due to storage in the ice bath has been seen.

RESULTS

Catheters were implanted into the aorta and renal vein in twelve dogs by the techniques described. It was possible to maintain patency of the catheters for a period of 10 or more days in eight of the dogs. These eight are reported upon here. Distinct increments in renal vein glucose concentrations were found. In the last four dogs studied, all prepared by method B, periodic X-ray confirmation of the position of the renal vein catheter was achieved by making films during the rapid injection of radio-opaque material into the catheter, at the end of the series of experiments.[11] In all cases, the position of the tip of the catheter and the path of the dye flow indicated that the catheter was in the renal vein.

Having established the surgical techniques, we proceeded at first to measure only the plasma arterio-venous differences. This was partly due to the initial assumption that this would accurately reflect the concentration differences in the whole blood. To check this point, both whole blood and plasma differences were studied in the last dog of the series, and, as is to be indicated, it appears that the increments in glucose concentration found in the renal vein are largely in the plasma fraction of the blood. Direct determinations of water content of the whole blood and plasma from the aorta and renal vein of this same dog have been made. Simultaneous measurements of the hematocrits of arterial and renal vein blood have been made in several dogs, and no measurable difference has been noted. This observation has been reported previously (23). Clearance of p-aminohippuric acid by each kidney has been measured in two dogs.

Table I presents the renal plasma arteriovenous differences measured in each of the successful preparations. All dogs were fasted for 16–20 hours before experiments. It is clear that in only one dog have we failed to see clearly positive average differences between renal vein and arterial plasma. The mean

[10] To correct for non-glucose reducing substances, the procedure described by Froesch and Renold (22) was used.

[11] The value of this procedure was emphasized by the discovery of a renal vein thrombosis in one dog, otherwise unsuspected. This dog is not included in the series reported.

TABLE I

Summary of Renal Vein-Artery (RV-A) Glucose Concentration Differences. (mg/100 ml)

Dog	Sex	Method of Prep'n	Post-operative Days Studied	Number Observations each Day*		Total Observations*		Mean RV-A ± S.E.	
				(PL)	(WB)	(PL)	(WB)	Plasma	Whole Blood
1 12 kg	M	A	2 6 7 9	2 2 2 1		7		+4.3 ± 1.43	
2 11 kg	M	A	2 6 9	1 1 1		3		+.9 ± .95	
3 16 kg	M	A	2	3 3		6		−.23 ± 2.12	
4 18 kg	M	A	2	4		4		+2.5 ± .83	
5 13 kg	F	B	2	5		5		+2.0 ± .32	
6 16 kg	F	B	7	5		5		+2.0 ± .31	
7 16 kg	M	B	3 8	4 1		5		+2.3 ± .66	
8 14 kg	F	B	8 15 22	5 4 4	6 7 5	13	18	+1.5 ± .21	+.97 ± .202

Mean RV-A (Plasma): All Dogs +1.9 ± .46 (S.E.)
Method A Dogs +1.9 ± 1.03
Method B Dogs +1.9 ± .18

(PL) = plasma; (WB) = whole blood

* This is the number of sample pairs drawn when the dogs were fasted for 16–20 hours. The data presented in this table were obtained at times when the arterial blood glucose levels were fairly constant, although sometimes they appeared to be rising slowly. The pairs were drawn at intervals of approximately 15 minutes. Typical blood and plasma glucose levels are shown in Table IV, a detailed presentation of data from one dog.

renal plasma glucose increment for the four dogs prepared by method B is presented separately, inasmuch as it seems likely that this is the superior method, and these dogs were kept in the jackets and frame during experiments. They were thus handled much less during the experimental periods. There is, however, no difference between this mean and the mean for all of the dogs. The mean whole blood glucose increment found in all samples drawn from dog 8 is also shown.

TABLE II

Comparison of Simultaneous Determinations of Plasma Glucose Concentration Differences in Renal Vein, Aorta and Inferior Vena Cava

Dog	Post-operative Day	Renal Vein-Aorta	Inf. Vena Cava-Aorta
5	2	+1.7 mg/100 ml	−1.0 mg/100 ml
		+2.3 mg/100 ml	−2.2 mg/100 ml
		+1.1 mg/100 ml	−2.3 mg/100 ml
		+2.3 mg/100 ml	− .6 mg/100 ml
		+3.0 mg/100 ml	−1.2 mg/100 ml
6	7	+2.3 mg/100 ml	−2.4 mg/100 ml
		+2.1 mg/100 ml	− .5 mg/100 ml
		+1.4 mg/100 ml	−1.2 mg/100 ml
Mean		+2.0	−1.4
S.E.		±.22	±.26

TABLE III

Extraction and Excretion of p-aminohippuric acid (PAH), dog 7

Collection Period	Urine Volume (ml.)		PAH Excretion (mg/min) (u × v)		PAH Extraction from Blood (Left) Per cent
	R*	L*	R	L	
1	25	25	5.50	3.12	43 per cent
2	61	50	3.05	2.81	55 per cent

* R = right. L = left.

This experiment was done 12 days after catheterization and 8 days after the bladder-splitting procedure (see Methods). When the dog was satisfactorily in place in the restraining frame, a priming dose of 17 ml. of 4 per cent sodium p-aminohippurate (pH 7) was given intravenously via an existing infusion in a leg vein, and was followed by a continuous infusion of .044 per cent sodium p-aminohippurate in .85 per cent NaCl at the rate of 7 ml/min., using a constant infusion pump. Forty minutes later, two urine collection periods, twenty minutes each, were started. Blood samples from the aorta and renal vein were drawn precisely in the middle of each collection period, for determination of PAH concentration. We are indebted to Dr. Floyd Rector for the PAH concentration measurements in the blood and urine samples. These were performed by the method of Bratton and Marshall as modified by Smith et al. (24).

In Table II are presented data on the plasma glucose arteriovenous differences in the two dogs in whom catheters were also placed in the inferior vena cava. In view of the sharp differences seen, little comment is necessary, other than that, even in this small series, the magnitude of the increase in glucose concentration in the renal vein plasma is statistically highly significant.

A comparison of renal function on the catheterized (left) and uncatheterized (right) sides is presented in Table III, performed in one dog. Details of this experiment are given in the legend. It is notable that the p-aminohippuric acid

(PAH) extractions on the left side are somewhat lower than one might expect, and that the PAH excretion on the left side is slightly lower than on the right. Urine flows, however, are nearly identical. In another dog, prepared by one of us (J. R. J.), using method B, and studied in an entirely similar manner, Rector and Orloff (25) have shown consistent PAH extractions in the catheterized kidney of about 75 per cent, which indicates good renal function. Equal PAH excretions by both kidneys were found. The left renal blood flow in this dog was calculated from the PAH excretion on the left side and the A-V difference, and was found to be 300 ml/min. This would indicate a total renal blood flow of 600 ml/min. The dog weighed 25 kg., and the total renal blood flow can thus

TABLE IV

Detailed Presentation of Blood and Plasma Glucose Concentrations in Dog 8

Post-operative Day	Glucose Concentrations (mg/100 ml)						Hematocrit
	Whole Blood			Plasma			
	Aorta (A)	Renal Vein (RV)	(RV-A)	Aorta (A)	Renal Vein (RV)	(RV-A)	
	80.5	83.0	+2.5	108.5	109.5	+1.0	
	82.0	83.5	+1.5	108.0	110.3	+2.3	
	81.7	81.3	−.4	107.3	108.0	+.7	40
8	—	—		105.8	108.3	+2.5	
	83.6	84.2	+.6	—	—		
	83.5	84.1	+.6	109.0	110.3	+1.3	
	84.6	85.4	+.8	—	—		
	76.0	77.7	+1.7	96.5	98.5	+2.0	
	76.4	76.2	−.2	98.8	99.8	+1.0	
	77.0	78.4	+1.4	98.0	98.3	+.3	
15	—	—		106.5	106.8	+.3	38*
	83.4	83.6	+.2	—	—		
	81.9	84.2	+2.3	—	—		
	84.2	84.2	0.0	—	—		
	85.9	86.1	+.2	—	—		
	—	—		93.5	95.5	+2.0	
	89.4	90.8	+1.4	118.8	121.0	+2.2	
22	93.0	93.7	+.7	126.3	128.3	+2.0	36
	98.3	99.1	+.8	131.5	133.3	+1.8	
	95.3	97.4	+2.1	—	—		
	96.4	97.7	+1.3	—	—		
Mean			+.97			+1.49	
S.D.				±.857		±.765	
S.E.				±.202		±.212	
P			<.001			<.001	

* This determination was made on post-operative day 18.

be expressed as 24 ml/kg. body weight/min. As will be shown in the discussion, this figure compares very favorably with other estimates of renal blood flow which have been reported by others.

Table IV presents a tabular summary of glucose concentrations in arterial and renal vein blood and plasma from the same pairs of bloods from dog 8. The mean whole blood difference in the series compared is +.97 mg. per cent, whereas the mean plasma difference is +1.49 mg. per cent. The ratio of the whole blood difference to the plasma difference ($\Delta WB/\Delta PL$) is thus .65. These figures suggest that the increment in glucose concentration in the renal vein above that in the artery is primarily in the plasma. It seems probable that the time period between drawing of the blood samples and their immersion in the ice bath (for plasma preparation) is too short to allow establishment of equilibrium between red cell and plasma water glucose concentrations. In a single experiment to test this point, it was shown that glucose added to chilled, aerated, dog jugular vein blood distributed itself in a volume close to that of the calculated plasma water volume. The glucose thus did not appear

TABLE V

Effects of Storage of Blood in Ice Bath on Plasma Glucose Concentrations

Storage Time (before centrifuging)	Measured Glucose Concentration		
	Arterial Blood (A)	Renal Vein Blood (RV)	RV-A
5 min	108.5 mg%	109.5 mg%	+1.0 mg%
85 min	108.0	108.8	+.8

Five ml. samples were drawn simultaneously from the aorta and renal vein, and each placed in an anticoagulant bottle. Following an initial chilling period, one half of each sample was centrifuged within five minutes of the time it was drawn, the other half being allowed to remain in an ice bath for 85 minutes. The plasma glucose concentrations found in each aliquot are shown, and it is apparent that no appreciable distortion of the renal vein-artery plasma difference has occurred.

TABLE VI

Water Content of Arterial and Renal Vein Whole Blood and Plasma in Dog 8 (gm/100 ml)

Post-operative Day	Whole Blood		Plasma		Hematocrit
	Artery (A)	Renal Vein (V)	Artery (A)	Renal Vein (V)	
15	86.48	86.25	94.60	94.25	38*
22	86.55	86.47	94.25	94.15	36
25**	86.30	86.20	93.70	93.40	33
Mean: A-V	Whole Blood .14 ± .047		Plasma .25 ± .076		

* Hematocrit done on the 18th post-operative day. On day 8 it was 40.

** The dog had been on cortisone acetate 5 mg/kg I.M. for four days as part of an initial experiment not reported here.

to enter the red cells to an appreciable extent over a period of several minutes under these conditions. In another experiment it was shown that storage of paired arterial and renal vein blood samples in the ice bath for a period of 85 minutes did not appreciably affect the measurement of the difference in their plasma glucose concentrations. The concentrations in both samples dropped slightly, but to a nearly equal extent (Table V).

Determinations of the water contents of arterial and renal vein blood and plasma are shown in Table VI. These determinations were made by weighing known volumes of blood and plasma, followed by desiccation over P_2O_5 to constant weight. The average difference seen (Artery-Renal vein) was +.14 gm/100 ml. in whole blood and +.25 gm/100 ml. in plasma. These figures are quite reasonable in view of known renal blood flow rates and urine flow rates in dogs.

The significance of these findings is to be discussed.

DISCUSSION

It is clear that in these dogs, under the conditions described, the glucose concentrations in the whole blood and plasma leaving the kidney were higher than those in the whole blood and plasma of the aorta. As will be shown, these differences are greater than can be explained by hemoconcentration. It is also to be noted that these dogs were calm, unanesthetized and post-absorptive, and were undergoing no forced diuresis. They were also always at least two days into the post-operative period. There is no evidence from our data that the glucose increments seen in the renal vein diminished as the post-operative period lengthened.

That hemoconcentration cannot explain the concentration increments seen is indicated by the data in Table VI, and also by consideration of known rates of renal blood flow and urine flow in dogs. For example, Houck (26) has given values of 13.51 (±.375) ml/min/kg for the effective renal plasma flow, as determined by clearance of p-aminohippurate. From this figure, a total renal blood flow of approximately 23 ml/kg/min can be estimated. Cohn *et al.* (6), in their own studies of glucose synthesis by the kidney, measured renal blood flow by means of a rotameter implanted in the renal vein. The average figure for total renal blood flow (by our calculation) is 27.4 ml/kg/min (±2.5), in their dogs in the control periods. Thus, an average figure for total renal blood flow can be taken as 25 ml/kg/min in the dog. A 10 kg. dog would then have a renal blood flow of about 250 ml/min. If such a dog were to excrete 1 ml of urine a minute, which would be a very high flow rate in the absence of forced diuresis, the water loss seen in the renal vein blood would be 1/250, or .4 per cent. If no new glucose were delivered into the renal vein blood, but all the glucose in the renal arterial blood were present, one might expect a

maximum increment of .3 mg. per cent in the renal vein at the blood glucose concentrations seen.

In dog 8, in 18 pairs of whole bloods, a mean increment of +.97 mg. per cent was seen (Table IV). This figure is distinctly higher than that which could reasonably be expected from hemoconcentration, leaving a margin of at least +.6 mg. per cent as the smallest "new" glucose increment to be expected from our data.

It is notable that in dog 8 the mean plasma increment seen is lower than the mean for all the dogs. This suggests that the mean whole blood increment for all the dogs may also have been higher than that seen in dog 8. By taking the mean plasma increment of $+1.9 \pm .46$ mg. per cent (Table I) and multiplying it by the factor of .65 (the ratio of whole blood to plasma increments seen in dog 8, Table IV), estimates of +.935 to +1.53 mg. per cent can be made for the mean whole blood glucose increment. Subtraction of .3 mg. per cent, a maximal figure for hemoconcentration, would leave +.63 to +1.23 mg. per cent as the true whole blood glucose increment. This range of increments indicates a renal glucose production of 9.5 to 18.5 mg/kg body weight/hour, taking the total renal blood flow to be 25 ml/kg/min.

It is of some interest to compare this renal glucose production with previous estimates of hepatic glucose production in the dog. For example, Soskin and Levine (27) reported an estimate of 228 mg/kg/hour for the peripheral glucose utilization in the eviscerated dog. An estimate of hepatic glucose production can also be made by taking figures for hepatic blood flow (28) and hepatic A-V differences (29). Combination of these values leads to an estimate of 150 mg/kg body weight/hour in the well-fed, unanesthetized dog. Comparable estimates of hepatic glucose production in man have also appeared (30, 31). It seems that estimates of 150–200 mg/kg/hour are reasonable. The variations and uncertainties in estimating the magnitude of both renal and hepatic glucose production make further speculation at this point appear unprofitable, but from the figures available one might estimate a possible range for renal glucose production of 4.75 to 12.3 per cent of the hepatic. It seems unlikely, therefore, that renal glucose production is related specifically towards regulation of the blood glucose concentrations. On the other hand, it seems possible that renal gluconeogenesis may be related to some intrinsic activities of the nephron. In view of this possibility, further studies are being planned in which renal blood flow and glucose arteriovenous differences will be measured simultaneously by the techniques described, in a variety of situations. It is hoped that these studies will help to clarify this problem.

A final point of some interest remains to be discussed. In 1938, Bergman and Drury (1) demonstrated a distinct rise in the glucose requirements of the hepatectomized rabbit when the renal pedicles or the ureters were ligated.

In these animals, who had been previously fasted for four days, cessation of urine flow nearly doubled the rate at which it was necessary to infuse glucose in order to maintain normal blood glucose levels. It was concluded that the kidneys evidently removed from the blood a substance which in some way inhibited the glucose utilization of the peripheral tissues. The same type of observation has been made in experiments with eviscerated rats (2, 3). If it is possible to transpose data from rodents under such conditions to dogs under our conditions, certain questions may be raised. It is clear, for example, that if the rabbit kidneys after hepatectomy were producing no more than 12 per cent of the original hepatic output, it is not possible to explain the observations of Bergman and Drury on the basis of depriving the animals of renal glucose. Whereas this is highly speculative, it still appears to re-open the possibility that the kidneys exert some influence on peripheral glucose requirements. This possibility seems to be increased by the observation, already discussed, that ureteral ligation increases the apparent peripheral glucose requirements, while it does not seem to seriously interfere with renal glucose production. This matter deserves further investigation.

CONCLUSIONS

1) Previous reports of glucose synthesis by the kidney have been confirmed in the intact, unanesthetized dog, using a precise and highly specific glucose assay technique.

2) For these studies, techniques have been developed which allow the implantation of catheters into the aorta and renal vein several days prior to the definitive experiments.

3) Estimates of the magnitude of renal glucose synthesis have been made which indicate that the kidney produces blood glucose at a rate of 9.5 to 18.5 mg/kg body weight/hour. This is estimated to be in the broad range of 4 to 13 per cent of the hepatic glucose production.

4) The possible implications of this observation are discussed.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY IN ASSOCIATION WITH THE ALPHA OMEGA ALPHA FRATERNITY

May 12, 1958

HISTOCHEMICAL DEMONSTRATION OF CYTOCHROME OXIDASE WITH A NEW REAGENT FOR THE NADI REACTION

DAVID T. CRAWFORD

Paper to be published in its entirety.

COMPARISON OF INTRADERMAL AND INTRAMUSCULAR IMMUNIZATION OF MICE WITH FLUID TETANUS TOXOID

HAROLD C. NEU AND JAMES H. NORTON

In an attempt to investigate the comparative efficiency of intradermal and intramuscular immunization, Webster-Swiss white mice were injected with fluid tetanus toxoid. The antitoxin response was assayed by challenge with tetanus toxin. Animals were immunized with 0.1 ml. of toxoid in doses ranging from 0.15 to 2.4 Lf (0.0125–0.2 ml.) and challenged with toxin doses of 2 and 5 MLD at 7, 14, 21, and 30 days after a single initial immunizing dose.

The intradermal route at 7, 21, and 30 days after the initial injection was equivalent to but never significantly better than the intramuscular route. The antitoxin response at 14 days was maximal for both routes. The intradermal route of injection was significantly better than the intramuscular route at antigen levels of 1.2 and 0.3 Lf. Mice immunized intradermally generally became paralyzed at a much slower rate or died later than did the intramuscularly immunized groups. Combining all the immunizing doses and both challenge levels given at 14 days after the primary injection, it was found that intradermal immunization resulted in better protection against paralysis at the 3 per cent level of confidence and against death at the 1 per cent level.

These results were confirmed in another experiment comparing the effect of two immunizing doses. Animals were immunized with 0.3 Lf (0.025 ml.) of fluid tetanus toxoid and were given another injection 30 days later. The best protection was observed when both injections were intradermal. Mice immunized in this manner showed less paralysis than animals given two intramuscular injections. The difference was significant at the 1 per cent level of confidence. The preliminary data indicate that those animals which received the initial injection intradermally always had significantly better protection than those receiving the initial injection intramuscularly regardless of the route used for the second injection. These data further suggest that initial intradermal immunization may be important in determining the extent of subsequent antibody production.

Although it appears that the intradermal route of immunization may be superior to intramuscular immunization in mice, these results cannot yet be extrapolated to the human situation since the mouse does not necessarily produce antibody in a fashion identical with the human being, and the possibilities of allergic reactions that may result from intradermal injections were not investigated.

INTERACTION OF INFLUENZA VIRUS AND VASCULAR ENDOTHELIUM AS DETERMINED BY PERFUSION OF THE PORTAL VEIN OF THE MOUSE

GEORGE H. KESSLER, JR.

Mice injected intravenously with massive doses of influenza virus die with peritoneal hemorrhages and hepatic necrosis. These pathological findings suggested that the virus may affect vascular endothelium. Ivanovics, *et al.* presented evidence for interaction of influenza virus and endothelium of the excised formalinized liver perfused through the portal vein by gravity flow. However, the errors inherent in their methods, and the inability to reproduce their results led to a modification of the perfusion technique. Polyethelene catheters were inserted into the portal veins and inferior vena cavae of mice and the livers perfused *in situ* by means of an infusion pump set to deliver at a constant rate. Therefore, a quantitative gradient of viral hemagglutinin across the hepatic vasculature could be plotted against a constant rate of virus flow.

Accurate, reproducible results were obtained by this method. The WS strain of influenza A virus adsorbed on hepatic endothelium at temperatures of 4° and 22°C., and eluted spontaneously at 37°C. The percentage uptake of virus appeared to be independent of the perfusion rate and concentration of virus. The vascular receptor sites of the normal liver could not be saturated even when perfused with 350,000 hemagglutinin units of heated virus. In contrast, adsorption of staphylococcal bacteriophage was negligible. Further evidence of the specificity of the reaction was obtained by prior treatment of the hepatic endothelium with sodium periodate which destroyed the vascular receptors for influenza virus adsorption.

These studies indicate that the vascular endothelium is a potential site for the toxic action of influenza virus.

EXPERIMENTAL STUDIES WITH GANTRISIN AND KYNEX IN TREATMENT OF PYELONEPHRITIS

BASHIR A. ZIKRIA

To be published in the September issue.

THE EFFECT OF POTASSIUM DEFICIENCY ON TUBULAR TRANSPORT OF GLUCOSE AND AMINO ACIDS

HARRY BESKIND

In order to evaluate the effect of potassium deficiency on the renal tubular transport of glucose and amino acids, experiments were performed on two trained female dogs 1) under control conditions, 2) during potassium depletion produced by desoxycorticosterone (DCA), and 3) after potassium repletion produced by increased potassium intake while still maintained on DCA. Standard techniques for measurement of maximal tubular transport of glucose (Tm_G) were employed. Spontaneous amino acid excretion was determined on casual samples of urine with the use of two-dimensional paper chromatography. The typical course of potassium depletion was established by the cumulative balance data and serum electrolyte concentrations.

The results showed that potassium deficiency *per se* had no effect on the glomerular filtration rate or Tm_G. The amino acid excretion patterns showed little or no change from the control specimens, except that one sample obtained during the potassium deficient state showed complete absence of lysine and arginine. No increase in aminoaciduria was detected.

Neither hyperglycemia nor spontaneous glycosuria were detected throughout the study. During the determination of Tm_G glucose assimilation (the difference between the rate at which glucose was infused and its rate of excretion in the urine) was markedly decreased in the potassium deficient state. It was thought unlikely that this change was due to simple starvation, because the animals consumed all of their food up to the time these observations were made. Glucose assimilation returned towards normal levels during the repletion period. It is suggested that potassium deficiency itself has a direct effect on overall carbohydrate metabolism.

THE RELATION OF SUTURE-LINE TENSION TO THE HEALING OF TRACHEAL ANASTOMOSES: APPLICATION TO THE REPAIR OF EXPERIMENTAL TRACHEAL DEFECTS

J. Roland Folse

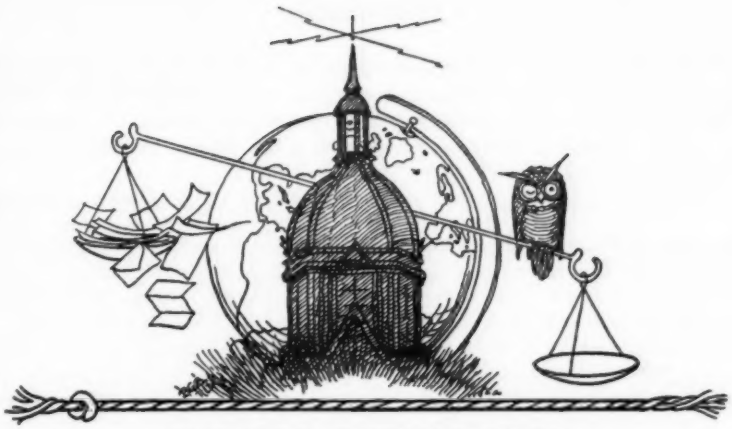
Serious difficulties are associated with the use of prosthetic materials and the various grafting techniques advocated for the repair of circumferential tracheal defects. On the other hand, when direct end-to-end suture is possible, the results are highly satisfactory. It is, therefore, important to determine the limits of feasibility of direct anastomosis, preferably in terms of a parameter which will allow the application of experimental results to the human.

A series of seventeen dogs have had segments of cervical trachea resected, creating defects of from 8–27 rings. The normal canine trachea consists of about 40 rings. The relationship between length of defect and resulting suture-line tension has been determined and a correlation established between suture-line tension and anastomotic healing or disruption. Tension was measured with the neck flexed by two spring balances.

Depending upon the length of the defect, suture-line tension varied from 500 to 2750 grams. Seven dogs with a suture-line tension of 1800 grams or less had good healing of the anastomosis without stenosis. In the range of 1800–2100 grams tension, three of six dogs healed satisfactorily, while the remaining three disrupted. Above 2100 grams tension one animal survived while three died due to disruption of the anastomosis.

Suture-lines were made with interrupted sutures of braided steel placed in the annular ligament which was left on the ends after transection.

While these results must be considered preliminary, it is apparent that if properly sutured, the trachea will heal satisfactorily under much greater tension than has previously been recognized.



BOOK REVIEWS

Principles of Research in Biology and Medicine. By DWIGHT J. INGLE. 123 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa.

In this slim book a wise professor tries to set down for students preparing for research careers in biology and medicine some of the general principles that should govern their work. He does so with grace. Aided by occasional bits from Lewis Carroll, G. K. Chesterton, and others, Dr. Ingle leads his reader through discussions of such diverse topics as the aims, methods, and assumptions of science, the virtues of parametric research, and the complexities surrounding the concept of causality. He also includes a balanced (but too brief) account of extrasensory perception. A recurring theme concerns the many ways in which a scientist may come to grief on his way toward establishing a fact. Pointed illustrations from the literature abound.

A minor criticism should be made. It is hard to believe that any reader who found himself on page 95 would need to be told how to calculate a mean or that perfect positive correlation is represented by +1. Fortunately, this type of misjudgment of the probable sophistication of the audience is rare and does not detract from the book's success.

VICTOR G. LATIES

Polysaccharides in Biology. Transactions of the Second Conference April 25, 26 and 27, 1956. Edited by GEORG F. SPRINGER. 245 pp., $5.00. Josiah Macy, Jr. Foundation, New York, N. Y.

Despite its rather broad title, this little book covers three specific subjects only: (1) The chemistry of sialic acid and related compounds; (2) The interaction between certain polysaccharides and a few viruses; and (3) The chemistry and pharmacological effects of bacterial pyrogens. These rather limited topics were chosen no doubt because there have been recent advances in each field that have created considerable general interest. The contents of the book are an almost stenographic record of a conference of 25 experts in these three not very closely related fields held in April, 1956, under the auspices of the Josiah Macy, Jr. Foundation. The proceedings were informal and the description of any individual's work was interrupted many times for questions or comments. A transcription of such a discussion is not likely to be an integrated review designed to inform the uninitiated. On the other hand, for workers in the field it will be a stimulating record of the unrehearsed views of the leading

experts. Furthermore, many disciplines are represented and the thoughts of the participants from related fields are often of interest even though they are occasionally naive. I am sure that the greatest value of this conference was for those actually present, nevertheless, there is some purpose in allowing interested parties to sit in at a distance by publishing the proceedings in an unedited form.

PHILIP S. NORMAN

Tuberculosis in White and Negro Children. Vol. I. The Roentgenologic Aspects of the Harriet Lane Study. By JANET B. HARDY. 122 pp., $7.50. **Vol. II.** The Epidemiologic Aspects of the Harriet Lane Study. By MIRIAM E. BRAILEY. 103 pp., $4.50. Published for the Commonwealth Fund by the Harvard University Press, Cambridge, Mass.

These volumes describe in detail the methods used and findings observed in a study of more than 1000 tuberculous children followed in the Harriet Lane Home Pediatric Clinic of the Johns Hopkins Hospital. The investigation was begun in 1928 and concluded in 1950. All subjects were included in the study prior to the era of specific chemotherapy for tuberculosis. Vol. I is a fine collection of serial roentgenograms and considers the various radiologic features of pulmonary tuberculosis. The anatomic characteristics of the normal chest are presented for orientation, and a number of conditions other than tuberculosis are shown to illustrate some of the difficulties in differential diagnosis. There is a final section of this volume on bronchoscopy and bronchography written in collaboration with Dr. Donald F. Proctor. Although a few bronchograms are illustrated in the roentgenograms the rationale for including this final section is not clear. It might have been more in keeping with the general theme if photographs of bronchoscopic abnormalities could have been inserted.

Vol. II is an extensively detailed analysis of the patients included in the study. A wealth of data is presented comparing the features of tuberculosis in white and negro children. The volume is divided into two major sections; one on "The Prognosis of Tuberculous Infection in Children" and another on "The Risk of Developing Reinfection Pulmonary Tuberculous. . . ." . A simple conclusion derived from this study, already well recognized, is that Negro children handle tuberculosis less well than do white children, even when both groups are living under similar socio-economic conditions.

LEIGHTON E. CLUFF

The Neuroses and their Treatment. By EDWARD PODOLSKY in collaboration with Seigfried Fischer, Montague Ullman, Frank C. Metzger, Max M. Stern, Henry H. Brewster, Paul Chodoff and L. J. Meduna. 555 pp., $10.00. Philosophical Library, New York N. Y.

The title of this book can be misleading. It is a collection of 37 articles previously published in the literature. The subject matter and biases of the authors represented vary as sharply as the merits of the individual articles. Apparently the editor attempts to convey a comprehensive view of the neuroses and their treatment. Instead he presents a hodgepodge of papers having no unity in theoretical foundation. No discernible limits in the topics selected plus a looseness of organization make the book's value questionable even as a reference volume.

The work perhaps serves one useful purpose. It reminds us that a lack of fundamental agreement exists in psychiatric conceptual and treatment approaches, but of this serious students hardly need to be reminded. The editor might have contributed more had he furnished us with a bibliography or bibliographies organized to cover more definite aspects of the neuroses.

This book has the potential for establishing more confusion than clarification for those

uninformed who may be misled to believe the comprehensiveness implied in the title "The Neuroses and their Treatment".

WILLIAM D. WHEAT

BOOKS RECEIVED FOR REVIEW

Annual Review of Medicine, Vol. 9, 1958. Edited by DAVID A. RYTAND. 530 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

Applied Physiology of the Eye. By H. WILLOUGHBY LYLE assisted by T. Keith Lyle. 341 pp., $9.00. Baillière, Tindall and Cox, London, England. The Williams & Wilkins Co., Baltimore, Md. exclusive U. S. agents.

Biophysical Principles of Electrocardiography, Vol. 1. By ROBERT H. BAYLEY. 237 pp., $8.00. Paul B. Hoeber, Inc., New York, N. Y.

Care of the Premature Infant. By EVELYN C. LUNDEEN AND RALPH H. KUNSTADTER. 367 pp., $8.00. J. B. Lippincott Co., Philadelphia, Pa.

Clinical Enzymology. Edited by GUSTAV J. MARTIN. 241 pp., $6.00. Little, Brown & Co. Boston, Mass.

Diagnostic Medical Parasitology. By EDWARD K. MARKELL AND MARIETTA VOGE. 276 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

Family-Centered Maternity Nursing. By ERNESTINE WIEDENBACH. 345 pp. G. P. Putnam's Sons, New York, N. Y.

Genetic Resistance to Disease in Domestic Animals. By FREDERICK BRUCE HUTT. 198 pp., $3.50. Cornell University Press, Including Comstock Publishing Associates, Ithaca, N. Y.

Glaucoma. Transactions of the Second Conference, Dec. 3, 4 and 5, 1956. Edited by FRANK W. NEWELL. 245 pp., $4.95. The Josiah Macy, Jr. Foundation, New York, N. Y.

Hermaphroditism, Genital Anomalies and Related Endocrine Disorders. By HOWARD W. JONES, JR. AND WILLIAM WALLACE SCOTT. 456 pp., $16.00. The Williams & Wilkins Co., Baltimore, Md.

Home Conditions. A Sociomedical Study of 1066 Hospitalized Patients with Skin and Venereal Diseases. By ESBERN LOMHOLT. Reprint from Acta Dermato-Venereologica. 100 pp., Dan. kr. 14.00. Rosenkilde and Bagger, Copenhagen, Denmark.

L'Auto-Intoxication apres Brulure. By T. GODFRAIND. 191 pp. A Christiaens, Bruxelles, Belgium.

Modern Treatment Yearbook 1958. Edited by SIR CECIL WAKELEY. 312 pp., $6.00. Published for The Medical Press by Baillière, Tindall & Cox, London, England. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Florence Nightingdale and the Doctors. By SIR ZACHARY COPE. 163 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

The Preservation of Youth. Essays on Health. By MOSES MAIMONIDES. Translated from the Original Arabic (Fi Tadbir as-Sihha). 92 pp., $2.75. Philosophical Library, New York, N. Y.

Regional Ileitis, 2nd revised edition. By BURRILL B. CROHN AND HARRY HARNIS. With Special Contributions by Richard H. Marshak and David A. Turner. 239 pp., $7.25. Grune & Stratton, New York, N. Y.

Rehabilitation of the Cardiovascular Patient. By PAUL DUDLEY WHITE, HOWARD A. RUSK, PHILIP R. LEE AND BRYAN WILLIAMS. 176 pp., $7.00. McGraw-Hill Book Co., New York, N. Y.

Safety Techniques for Radioactive Tracers. By J. C. BOURSNELL. 68 pp., $1.75. Cambridge University Press, New York, N. Y.

A Search for Man's Sanity. The Selected Letters of Trigant Burrow with Biographical Notes. Foreword by Sir Herbert Read. 615 pp., $8.75. Oxford University Press, Fair Lawn, N. J.

Skin Grafting. By JAMES BARRETT BROWN AND FRANK MCDOWELL. 411 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

The Student Life. The Philosophy of Sir William Osler. Edited by RICHARD E. VERNEY. With forewords by John Bruce and Alec H. Macklin. 214 pp., $4.00. The Williams & Wilkins Co., Baltimore, Md.

THE INFLUENCE OF TOTAL BODY X-IRRADIATION ON THE RESPONSE OF MICE TO METHYLCHOLANTHRENE[1]

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It is now well established that exposure of the whole body to ionizing radiations is followed by physiological changes, that, with dosages below the immediately lethal range, result in a variety of morbid conditions to which the organism eventually succumbs. The mechanisms by which these morbid states are brought about are still not understood or only poorly apprehended. Notable among the various pathological states, and among the easiest to recognize, are tumors, and these have attracted a great deal of attention ever since the carcinogenic properties of ionizing radiations were recognized more than fifty years ago. Other late lesions, less dramatic, perhaps, but of importance nevertheless, include abnormalities of the blood-forming and reproductive organs, cataracts, graying of the hair and vascular changes, among others. Some of these lesions are truly induced entities, i.e. they are entities unlikely to occur in this form during the lifetime of the unirradiated individual, whereas others represent abnormalities that occur at an accelerated rate in the exposed individuals as compared with unexposed ones; not only do they occur earlier, but they also appear in a larger proportion of the animals and they are of greater severity. However, regardless of whether they are "induced" or whether they represent an acceleration of naturally occurring pathological conditions, they generally lead to premature death. All of the abnormalities that follow sublethal exposure to ionizing radiations characteristically have latent periods or incubation periods of variable length, a state of affairs that is familiar from other diseases. This feature of the long latent period is again most strikingly illustrated in the case of radiation-induced tumors, but it is also characteristic of most of the other abnormalities mentioned earlier. Since the nature of the physiological and cellular derangements that take place during these long silent phases is largely obscure, it is very difficult to obtain a clear picture of the natural history of these diseases.

The following experiment was designed, therefore, to define one small parameter of the complex biological situation resulting from radiation injury

[1] Work performed under the auspices of the U. S. Atomic Energy Commission.

[2] Former member of staff of the Department of Pathology, Johns Hopkins University School of Medicine.

to the whole organism, and to attempt to explore the subtle changes that must occur in the internal environment during the latent phase. To this end, we undertook to test the ability of irradiated animals to react to a chemical carcinogen and thus to measure the degree of susceptibility to the development of induced tumors.

Experimental procedures

The experiment was designed to compare the induction time and yield of tumors produced by methylcholanthrene in two groups of animals which were identical in all respects with the exception that one was irradiated and the other was not. It has been shown by several investigators (1, 2) that age may influence the production of induced tumors in mice, and Saxen (3) recently demonstrated that the total incidence and the length of the induction time of mortus induced by the subcutaneous injection of methylcholanthrene in mice was a function of age, in that young animals developed tumors more readily and with a shorter induction time than did old animals. This age factor promised to be a useful experimental tool in the study of the present problem. Since a change in susceptibility of the irradiated animals might be in the direction of either younger or older animals, it was decided to select middle-aged animals for the two crucial experimental groups and to bracket them with a group of young animals on one side and with another group consisting of much older animals on the other side.

C_3H male mice, purchased from the Jackson Memorial Laboratory, Bar Harbor, Maine, were collected over an appropriate period of time such that four main groups of animals with the following age distribution were available for the injection of methylcholanthrene: I, Young animals, 4 months old; II, Middle-aged, 8.5 months old; III, Middle-aged, 8.5 months old and irradiated 5.5 months previously, at the age of 3 months; IV, Old animals, 14 months of age. In addition, there were two groups of 41 animals each, one consisting of irradiated animals injected with tricaprylin, the solvent for the methylcholanthrene, and the other comprising irradiated animals that were not injected at all.

When three months old, 178 animals were given 500 r of x-rays from a Maximar Unit, operated at 250 KVP with added filtration by 5 mm. Cu. and 3 mm. bakelite; half value layer of 1.6 mm. Cu. The dose rate was 37 r/minute. The mortality from this exposure was only 4.5 per cent in 30 days, and, since it seemed desirable to study the effect of a rather high dose of radiation, it was decided to give a second dose of x-rays. Therefore, an additional 250 r was given to the survivors 5 weeks after the first exposure. The mortality was only 5.3 per cent during the next 142 days; thus the overall mortality following this fractionated irradiation was 10 per cent in 176 days.

An interval of five and one half months between irradiation and injection of carcinogen was chosen, to afford the animals ample opportunity to recover from the initial injury and to progress into the latent phase of subsequent abnormalities of specific organs or organ-systems. On the other hand it was necessary to make the interval between irradiation and injection of the carcinogen not too long in order to have relatively healthy and not debilitated animals for experimentation. For these and other more practical reasons five and a half months seemed a reasonable interval.

Methylcholanthrene (Eastman Kodak white label), through the kind cooperation of Dr. J. H. Pomeroy of this Laboratory, was recrystallized first from isopropyl alcohol and then from acetone-water; the final melting point was 181.8°C. (Kofler hot stage). The identity of the material was ascertained by ultraviolet absorption spectroscopy; it was stored in the dark to prevent formation of photo-peroxides. Shortly before use, 10.4 mg. of methylcholanthrene were dissolved in 69 cc. of tricaprylin. Of this solution 0.2 cc., containing 0.03 mg. of methylcholanthrene, were injected into the subcutaneous tissue of the right axilla, slightly anteriorly, through a 22-gauge, 1.5-inch needle which, as a precaution, was inserted through the skin of the right groin, in order to minimize leakage. The dose level of 0.03 mg. of methylcholanthrene was chosen because it seemed desirable to use a dose that would produce tumors in not more than 60 to 80 per cent of the animals at about 200 days. It was thought that this would make it possible to detect differences in susceptibility more readily than by use of an overwhelming dose of carcinogen that would be 100 per cent effective in a short period of time. The data of Saxen (3) and of Bryan and Shimkin (4) indicated that 0.03 mg. would be the appropriate dose level.

Following the injection of methylcholanthrene, all animals were examined at frequent intervals, and special emphasis was placed on the establishment of the diagnosis of a malignant tumor at the site of injection. The animals were housed in plastic cages, eight to a cage. Water and Rockland mouse diet were supplied *ad lib*.

RESULTS

General

With the exception of slight initial mortality, no clinical evidence of illness was noted among the surviving irradiated animals during the first five months after exposure.

Prior to the injection of the methylcholanthrene, a moderate degree of graying of hair was noted in most of the irradiated animals. Within 13 months after exposure, all survivors had a completely or almost completely white hair coat. The pattern of graying was not uniform, as can be seen from Figures 1 and 2. It progressed in stages and in a manner identical with that described by Chase (5). As pointed out by this author, these patterns are related to cycles of hair growth at the time of irradiation. There was no change in hair color in the nonirradiated mice, although the hair coat was slightly thinner in the old animals of Group IV. Cataracts began to appear in the irradiated animals about one year after exposure, at a time when the graying of the hair was almost complete. The cataracts usually were bilateral and the opacities of the lenses, once present, increased rapidly in extent. No cataracts have been observed in any of the nonirradiated animals of any age group. Sixteen to eighteen months after exposure all surviving animals were thin and emaciated and had gray hair and bilateral cataracts. They wasted away and most of them died with infections either of the lungs, or, more frequently, of the intestinal tract. The testicles, at the time of autopsy, showed extreme atrophy in all animals dying a year after exposure or later. The spleen, lymph nodes and thymus similarly showed extreme involution. Although a few miscellaneous tumors were observed among the irradiated animals, such as lymphoma,

hepatoma and carcinoma of the skin, the same number (five) of almost identical tumors was seen in the nonirradiated group. Unfortunately, the animals were not weighed during the first nine months after x-ray exposure. However, when weights were taken at this time, there were no noteworthy differences between the irradiated survivors and the nonirradiated animals of the same age group, although old animals at this time weighed a few grams less, on the average, than the animals in the other three groups.

Following the injection of methylcholanthrene in tricaprylin, or of tricaprylin alone, most animals showed a transient epilation and superficial ulceration of the skin overlying the injected material; this healed within two or three weeks. Later on, the abnormalities that were revealed by palpation of the subcutaneous tissue could be conveniently classed into five categories, of which the last two represented positive evidence of a malignant tumor in the form of small and firm nodules or sizeable tumor masses. The first unequivocal tumors were found 69 days after injection; there were seven animals with tumors at this time and they were found in three of the four groups. This interval of 69 days thus constituted the minimal induction time of tumors, and, for purposes of calculation of tumor rates and total incidence, only those animals were used that were alive on the 69th day after injection, namely, 76 animals in Group I, 79 in Group II, 76 in Group III, and 60 animals in Group IV.

Tumor incidence and induction time

The tumor data, as collected from interval to interval, are recorded in detail in Table I. The cumulative incidence of tumors is plotted for each of the four groups in Text-figure 1. The ordinate shows the animals with tumors as per cent of animals alive on the 69th day after injection, and the abscissa the time interval in days following injection. It is evident that there are both similarities and differences in both total incidence of tumors and in the duration of the induction time among the four groups. There was a sharp rise in incidence of tumors among the young animals and among the middle-aged, nonirradiated animals between the 97th and 143rd day after injection, and the two curves parallel each other closely during this interval. During the same interval, there was a similar parallelism between the middle-aged, irradiated, animals and the old ones, both of which were lagging somewhat behind the two former groups; this relationship was maintained by all groups until the 129th day, when the irradiated animals began to deviate from the old ones by showing an acceleration of tumor formation. The incidence of tumors among the irradiated animals subsequently remained between that of the unirradiated and of the old animals until the end of the experiment. The difference in the cumulative tumor incidence between the two middle-aged groups was significant at the 1 per cent confidence level for the observation periods from the

TABLE I

Tumors Detected by Palpation at Various Intervals Following Subcutaneous Administration of Methylcholanthrene to Four Groups of C_3H Male Mice

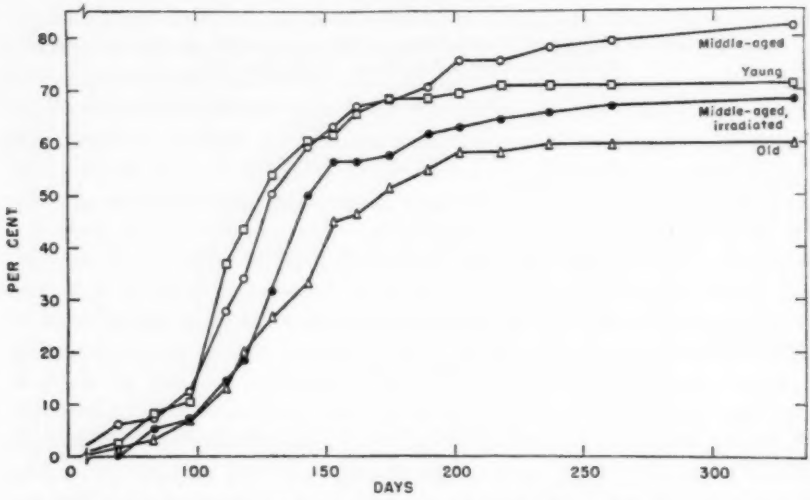
		End of Interval (in days following methylcholanthrene injection)																
		69	83	97	111	118	129	143	153	162	175	190	202	218	237	261	331	586‡
Group I (4 months old) nonirradiated	At risk*	76	74	70	68	47	41	33	27	26	23	20	20	18	16	15	15	12
	New tumors	2	4	2	20	5	8	5	1	3	2	0	1	1	0	0	0	0
Group II (8½ months old) nonirradiated	At risk	79	74	72	68	55	50	37	30	27	24	21	19	14	13	11	10	7
	New tumors	5	1	4	12	5†	13†	7	3	3	1	2	4	0	2	1	2	0
Group III (8½ months old) irradiated	At risk	76	76	72	70	63	59	48	33	25	24	23	20	17	15	12	9	3
	New tumors	0	4	1	6	3	10	14	5	0	1	3	1	1	1	1	1	0
Group IV (14 months old) nonirradiated	At risk	60	59	58	52	45	40	35	25	16	14	11	8	5	5	3	3	1
	New tumors	1	1	2	4	4	4	4†	7	1	3	2	2	0	1	0	0	0

* At risk = Number of experimental animals alive without tumors at end of previous interval.
† Includes mouse dying during interval, with tumor first discovered at autopsy.
‡ Alive (without tumors) after 586 days: Group I, 4; II, 2; III, 0; IV, 0.

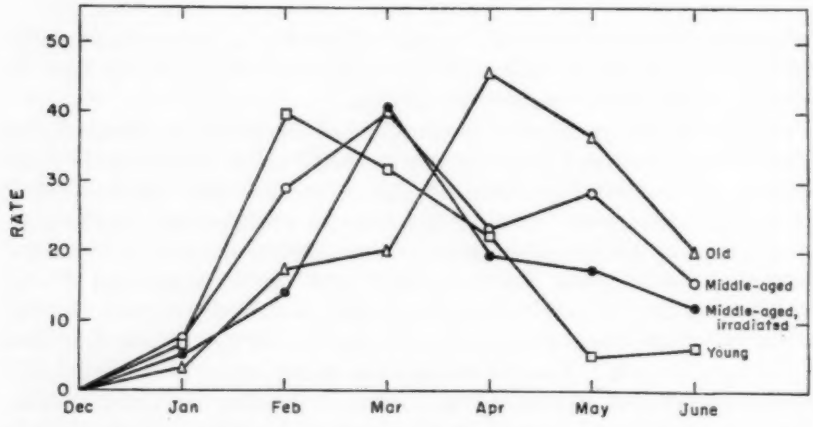
97th through the 129th day after injection. Thereafter, as seen in Text-figure 1, the incidence in the irradiated group increased more rapidly, so that the difference between the two groups diminished.

At 175 days the young animals virtually had reached the maximum tumor incidence of 71 per cent and only two additional tumor animals were found between 175 and 586 days. Similarly, the old animals also began to level off at 200 days and only one additional tumor animal was found after this interval. Both groups of middle-aged animals, on the other hand, continued to show a perceptible, albeit slight, rise in incidence until the 331st day, and it is of interest that the irradiation did not suppress this apparently innate capacity of the middle-aged animals to form additional tumors beyond 200 days. It is of further interest that the total incidence of tumors among the nonirradiated, middle-aged animals was slightly higher than that among the young animals. This was unexpected in view of the observations of Saxen and others that the very young animals show the greatest incidence of tumors.

The initial delay in the induction time of tumors in Groups III and IV is again apparent when the data are plotted in terms of monthly tumor rate, i.e. tumors occurring in animals at risk during each 30-day period, as shown in



TEXT-FIG. 1. Cumulative Tumor Incidence. Ordinate indicates total tumors detected to date by palpation or on autopsy, as per cent of the animals alive 69 days after injection, the first examination date at which tumors were found in any group. Abscissa indicates days after injection (Nov. 1, 1955). Young, 4 months old:-□——□-; Middle-aged, 8½ months old:-○——○-; Middle-aged, 8½ months old, X-irradiated 5½ months prior to injection:-●——●-; Old, 14 months old:-△——△-.



TEXT-FIG. 2. Monthly Tumor Rate. Ordinate indicates tumors detected by palpation or on autopsy during each month as per cent of the animals alive and without tumors at the beginning of each month.

TABLE II

Tumors in C_3H Male Mice Following MCA Injection

Age when Injected (months)	Authors	No. of Mice	Total Crude Incidence	Incidence at 120 Days	Time to 50 Per Cent Incidence	Median Time for Induction
			per cent	*per cent*	*days*	*days*
2–3	Bryan and Shimkin	20	65	33	140	120
3	Saxen	55	93	48	120+	117
4	Present	76	71	45	125	110
8.5	Present	79	82	35	132	125
8.5 (x-ray)	Present	76	68	20	143	131
14	Present	60	58	21	175	140
12	Saxen	16	44	25	—	118

Text-figure 2. On the other hand, it is also evident from these curves that there are three distinct peaks, one characteristic for each age group; they differ from one another by intervals of one month and that of the young animals comes first, with a very steep rise. This is followed by those of the two groups of middle-aged animals which are identical except for their slopes. The peak of the old animals follows with an even greater delay, 2 months after the young and one month after the two middle-aged groups. The difference between the irradiated and nonirradiated middle-aged animals consists therefore in the slope of the curve of tumor rate rather than in the total cumulative tumor incidence. This implies that the tumor-producing factors within the irradiated animals assert themselves after an initial delay. The picture, therefore, is not a simple one and it reflects the sum of a variety of influences, largely unknown, that have a bearing on the development of induced tumors. It is very difficult and probably not very profitable to interpret the shape of the various curves following each peak in Text-figure 2, inasmuch as subsequent points are based on very few animals remaining in the experiment. For purposes of comparison the tumor data of the present experiment and those of Saxen and of Bryan and Shimkin are summarized briefly in Table II.

Pathology

The histopathological studies were restricted to examination of the induced tumors, and of obscure diseases occurring in these animals. A systematic study of all organs was beyond the scope of this investigation. The tissues were fixed in 10 per cent formalin or in Zenker formol (Helly's fluid). They were embedded in paraffin and all sections were stained with haematoxylin and eosin. Of the 207 induced tumors, 163, or 79 per cent, were examined histologically; these 163 tumors were almost equally distributed among the four groups of experimental animals. The tumors could be divided readily into five major types as follows:

Type 1. This group of 36 tumors (22 per cent) showed a considerable degree of differentiation and maturity of the cells. They exhibited a prominent fibrillar pattern of interwoven bundles of connective tissue fibres and collagen, often arranged in whorls. The cells were spindle-shaped and rich in cytoplasm; the nuclei were uniform and small, showing little variation in size or in shape. An especially noteworthy feature was the presence among the mature cells of numerous small, round hyperchromatic nuclei, the size of lymphocytes; these were surrounded on occasion by scant cytoplasm, but usually they were without clearly visible cytoplasm. Large tumor cells were seldom seen. Necrosis was always prominent and there was invasion of the surrounding normal tissues. This group of tumors has been designated as fibrosarcoma. It constituted the most differentiated group of tumors, whereas the following two types represent increasingly immature and anaplastic variants of it.

Type 2. There were 26 tumors in this group (16 per cent) and they were very similar to the first type, especially at low magnification. The fibrillar pattern was well developed and consisted of interlacing bundles of connective tissue fibres. However, at higher magnification the cells were irregular in size and shape and more atypical. The nuclei were still oval and vesicular, but larger on the average than in the previous group. The connective tissue fibres were plump, as if swollen, and closely packed together. The small, round hyperchromatic nuclei described earlier were found in only very few of the tumors in this group. Many tumor cells showed a distinct granularity of the chromatin, consisting of numerous tiny dots, which were very clearly demonstrable with the phosphotungstic acid-haematoxylin stain. This feature was not observed among the tumors of type 1. This group of tumors has been designated as spindle-cell sarcoma.

Type 3. This group of 53 tumors (33 per cent) showed the same fundamental fibrillar pattern as seen in types 1 and 2, but in addition they contained groups of very anaplastic cells. The latter showed bizarre, irregular nuclei with multiple nucleoli and large eosinophilic inclusion bodies. There were many multinucleated tumor giant cells, varying in number from tumor to tumor. There were scarcely any fibres in the more differentiated parts of the tumors. Necrosis was prominent. These tumors have been designated as pleomorphic sarcoma.

Type 4. There were 41 tumors in this group (25 per cent). They were radically different from the tumors in the first three groups and they were composed mainly of two kinds of cells. There were, in the first place, groups of relatively small, round cells with hyperchromatic nuclei and a moderate amount of cytoplasm that showed an intense acidophilia. These cells closely resembled myoblasts and they have been considered as such. The other component consisted of elongated and frequently anastomosing muscle fibres, containing one or more vesicular nuclei. These fibres usually stained red with the trichrome stain and

they also showed longitudinal striations. No cross striations were demonstrable. The diagnosis of this group of tumors was rhabdomyosarcoma.

Type 5. The fifth group of tumors comprised 7 specimens (4 per cent) which could not readily be fitted into any of the previous groups. They were very anaplastic tumors and two of them resembled liposarcoma. This group has been designated as anaplastic sarcoma.

The subdivision of the induced sarcomas into these five types could easily be repeated on subsequent blind readings of the slides and it did not seem to be an arbitrary procedure. Although we are aware of the shortcomings and limits of the so-called grading of tumors on histologic grounds, it nevertheless seemed of interest to study the distribution of these five types among the four groups of experimental animals. The results are shown in Table III. In columns 6 and 7 of Table III, the differentiated tumors of types 1 and 2 and the undifferentiated, highly anaplastic tumors of types 3, 4, and 5 have been combined and contrasted. This distribution shows that well over 50 per cent of the tumors among the young and middle-aged animals were anaplastic sarcomas, whereas the tumors of the old animals were predominantly of the more differentiated type (66 per cent), the exact opposite of the three other groups. Indeed, it may be of some significance that the irradiated animals showed the highest percentage of anaplastic tumors of all four groups. The results of this tumor analysis may be an indication that there were perhaps really only two major groups of animals in the present experiment, namely the old ones on the one hand and the middle-aged and young ones on the other, and that the changes produced by the irradiation in the middle-aged were relatively mild, despite the large dose.

Although measurements of the induced tumors were made at intervals or most animals, it has not been possible to correlate growth rate with tumor type. The mean induction times for the 5 tumor types were 132, 135, 134, 116, and 105 days respectively. None of the differences were statistically significant, however.

TABLE III

Distribution of Induced Tumor Types Among Experimental Groups

Experimental Group	Tumor Types										Differentiated (Types 1, 2)		Undifferentiated (Types 3, 4, 5)		Total No.
	1		2		3		4		5						
	No.	%	No.	%	No.	%	No.	%	No.	%	No	%	No.	%	
I. 4 months	7	14	8	16	16	33	15	30	3	6	15	30	34	69	49
II. 8½ months	16	30	5	10	15	29	13	25	3	6	21	40	31	60	52
III. 8½ months irradiated	5	13	5	13	18	47	10	26	—	—	10	26	28	74	38
IV. 14 months	8	33	8	33	4	17	3	12	1	4	16	67	8	33	24

DISCUSSION

In taking the response of mice to a single dose of methylcholanthrene as a measure of radiation-altered susceptibility to the development of induced tumors, it is perhaps surprising that the effect of a total dose of 750 r was not greater. A number of reasons may account for failure to demonstrate a greater effect. For instance, it is possible that the choice of the subcutaneous connective tissue as a test organ was not the most appropriate or advantageous for the purpose, even though it is known that radiation readily produces changes in the vascular tree, the cells and the intercellular substances of connective tissue (6). Again, it may be that the dose of methylcholanthrene was too large, masking effects that might have been revealed with a smaller dose, or that the time interval between irradiation and injection of the carcinogen was either too long or not quite long enough for an effect to become more strikingly demonstrable. Lastly, it is possible that the dose of 750 r simply was not large enough to bring about more radical changes in the susceptibility of these mice to this particular carcinogen. Evidently, these questions can be answered only by further experimentation.

However, the irradiation did produce severe lesions in the animals; some of them, such as graying of the hair, were already manifest at the time of injection of the carcinogen, whereas others did not become apparent until later. In any event, it was obvious that a sufficiently high dose of radiation had been administered to produce severe somatic effects in a number of organs, demonstrating again the classic syndrome of late radiation reaction. The other results of the experiment may be summarized as follows: there was a significant delay in the induction time of the tumors in the irradiated animals during the early phase and there was also a partial reduction in the total incidence of these tumors. These data can be interpreted to show that total body irradiation had indeed decreased the susceptibility of these mice to methylcholanthrene and that this decrease was beyond the statistical fluctuations often encountered in this type of experiment. Phrasing it differently, one could also say that total-body irradiation and methylcholanthrene, each in itself a carcinogenic stimulus, were not additive or synergistic under the conditions of this experiment. Although there was no evidence in this experiment that 750 r was in fact a sufficiently high dose to produce tumors *de novo* in C_3H male mice, nevertheless had there been synergistic action or additivity of radiation and methylcholanthrene, one would have expected a tumor yield in the irradiated animals at least as high if not higher than in the controls. On the contrary, however, there was, as has been seen, an inhibition of tumor formation by the irradiation. This inhibition is of special interest in view of the observations by Furth (7), for instance, who showed an enhancement by total-body irradiation with 125 r of the leukemogenic action of methylcholanthrene in Rf/Ak mice.

In the work of Furth and Boon, methylcholanthrene was painted repeatedly on the skin of the mice, and painting began immediately following x-irradiation; therefore the two experiments are not strictly comparable, but the results do demonstrate that x-irradiation may either enhance or inhibit tumor formation. It will require further experimentation to determine the mechanisms by which enhancement occurs in one case and inhibition in the other. Undoubtedly, the physiological conditions following immediately after exposure of the whole body to ionizing radiations, as in the case of the experiment by Furth, are radically different from those existing five or six months after exposure, as in the present experiment. Furthermore, it is certain that methylcholanthrene-induced leukemia in the Rf/Ak strain of mice is an entity different from sarcoma induced in the C_3H strain by the same material.

The transient retardation in tumor induction made the irradiated animals appear similar to old ones and the reduced tumor yield may be interpreted similarly. However, this resemblance was only superficial. The overall results of the present experiment show that the irradiated animals largely maintained their individuality and that their age-characteristic response to methylcholanthrene was altered only temporarily by prior exposure to radiation. Although the transient modification of this response was superficially similar to that seen in the old animals, it probably occurred for different reasons and not because the animals had been made to "age" prematurely. The pathological changes produced in these animals by irradiation in no way resembled those seen in the old animals who showed neither graying of the hair, cataracts, nor any of the other lesions that were observed in the irradiated group. The analysis of the histopathology of the tumors, with due regard for the limits of this procedure, also showed that the old animals were distinct from the other three groups.

Shortening of the life span as such, without specifying its causes, has been used extensively in recent years as a measure of assessing the effects of ionizing radiations in experimental animals, especially in the low dosage range. Unfortunately, this has been spoken of or equated with "premature aging" and it has also been referred to as the "aging effect" of ionizing radiations. While this may be useful for certain purposes, it is evident that the introduction of so vague a term as this is likely to do very little to clarify the nature of the biological phenomena that occur. Indeed, it will be of questionable value to do so as long as the term "aging" itself is little more than a collective term, denoting the numerous bodily changes associated with aging of the organism as a whole.

It may be concluded either that tumor induction by methylcholanthrene has not proven to be a very sensitive method for elucidating the nature of the changes produced in mice by total body x-irradiation, or that 750 r does not produce such changes to any marked degree in the connective tissue of the

skin of the mouse. On the other hand, it has been demonstrated that two carcinogenic stimuli are not necessarily additive and that irradiation may actually inhibit methylcholanthrene carcinogenesis in contrast to other studies in which an augmentation has been observed.

The present experiment has also shown that the abnormalities produced in animals by irradiation do not necessarily have much in common with those occurring in old age. The differences and possible similarities of morbid states characteristic of old age and of irradiation injury require careful analysis and sharp delineation; premature death due to irradiation is not to be confused with premature aging.

That age is an important factor in the induction of tumors by chemical carcinogens has again been demonstrated and the results of this experiment confirm and extend the findings of earlier investigators. The age-determined factors affecting carcinogenesis by methylcholanthrene in old mice are different from those operating in irradiated animals, although superficially the two may appear to be similar.

SUMMARY

Tumor induction with methylcholanthrene in C_3H male mice has been used as a means of determining the biological nature of the changes following total body x-irradiation. Such changes were expressed in terms of susceptibility to tumor formation in the subcutaneous tissue 5 to 6 months after x-ray exposure.

It was demonstrated that, although the irradiation produced a generally severe radiation reaction in the animals at the end of the experiment, the influence on susceptibility to induced tumors was only slight and transient. The change in susceptibility was apparent in a delay of the induction time and in a slightly lowered total incidence of the tumors. Total-body irradiation did not enhance carcinogenesis by subcutaneous methylcholanthrene, in contrast to the reported enhancement by irradiation of the leukemogenic action of methylcholanthrene paintings.

A concurrent study of the influence of age on susceptibility of mice to carcinogenesis by methylcholanthrene has shown that middle-aged animals and young animals are more susceptible to this material than old animals. Although the response to methylcholanthrene of irradiated animals was initially similar to that of old animals, there is reason to believe that the factors affecting carcinogenesis in old animals are different from those operating in the irradiated animal.

ACKNOWLEDGMENT

We wish to thank George Sacher for his advice in the design of the experiment and many helpful discussions during the course of the work. We are also grateful to Robert Schweisthal for the statistical analysis of the data. The technical assistance of Mrs. Mildred Summers and Mrs. Helen Cummins is gratefully acknowledged.

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PLATE I

FIG. 1. Irradiated animals one year after exposure. Nonirradiated control mouse of the same age group in middle. Graying is almost complete. × 0.67.

FIG. 2. One year after x-irradiation. Incomplete graying. × 0.88.

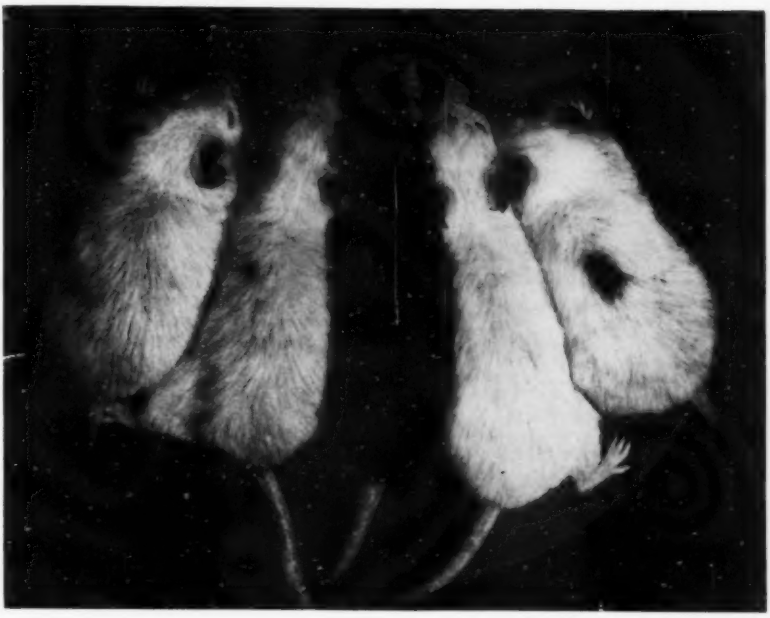

FIG. 1

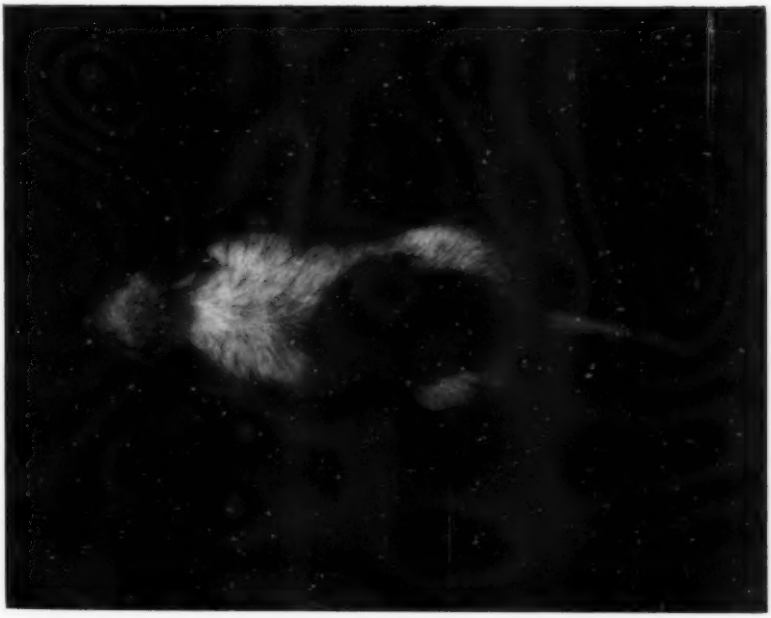

FIG. 2

THE RELATIVE IMPORTANCE OF BLOOD AND URINARY CONCENTRATIONS OF SULFONAMIDE IN THE TREATMENT OF URINARY TRACT INFECTIONS

A Comparison of Sulfisoxazole (Gantrisin) and Sulfamethoxypyridazine (Kynex, Midicel) in Man and Rat[1]

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Pharmacologic studies with sulfamethoxypyridazine[3] (Kynex,® Midicel®) in several species of animals have shown this sulfonamide to have a low renal clearance (1–3 ml./min. in dog (1) and man (2)). Experimental infections in mice (3) with a variety of organisms (*pneumococcus*, strain SVI; *streptococcus*, strain C203; *Klebsiella*, strain AD; and *Pasteurella*, strain 310) indicate that SMP and sulfisoxazole[4] (Gantrisin®) are about equally effective in preventing death from these infections when similar whole blood concentrations of the two drugs are attained. Such equal therapeutic effects can be achieved in mice by the oral administration of 5 to 8 times as large a dose of SXZ as of SMP because of the considerably higher renal clearance of SXZ. Accordingly, it was decided to compare these two sulfonamides in the treatment of urinary tract infections in the hope that the data obtained might permit some conclusion as to the relative importance of blood and urinary concentrations of drug in the treatment of such infections.

METHODS AND RESULTS

A. Clinical data

The first experiment was a controlled trial of SXZ and SMP in 31 male patients attending the Brady Urological Clinic because of urinary tract infection. The diagnoses included urethritis, cystitis, pyelitis, and pyelonephritis; often several of these entities were considered to be present in a given patient. In addition, it should be emphasized that approximately half of these patients were suffering from, or had recently been operated upon for, obstructive or calculous disease. Urinalysis and urine culture were obtained at the first visit,

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[2] American Drug Manufacturers' Association Fellow in Clinical Pharmacology.

[3] Sulfamethoxypyridazine is hereinafter referred to as SMP.

[4] Sulfisoxazole is hereinafter referred to as SXZ.

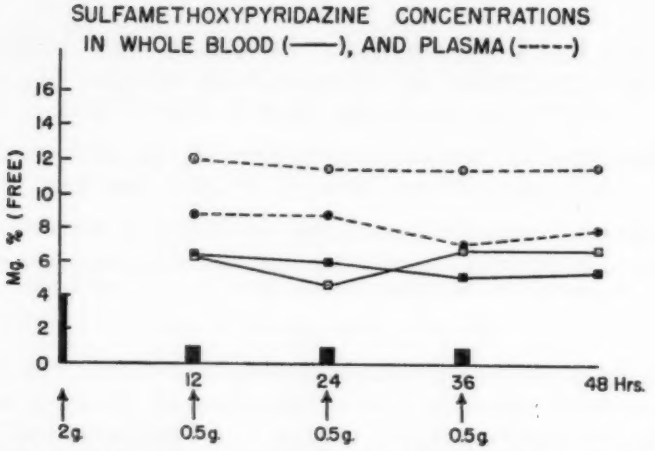

FIG. 1. Sulfamethoxypyridazine concentrations in two patients given a priming dose of 2 gm., followed by 0.5 gm. every 12 hours for 3 doses.

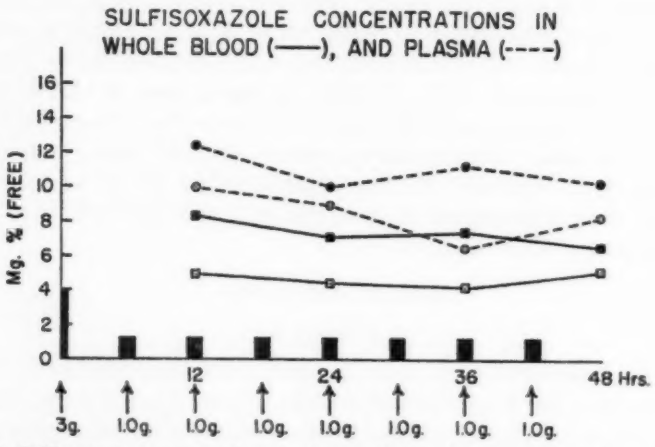

FIG. 2. Sulfisoxazole concentrations in two patients given a priming dose of 3 gm. followed by 1.0 gm. every 6 hours for 7 doses.

at which time symptoms were also elicited. Patients were allocated randomly to the two drugs. Preliminary data in 4 patients without genitourinary disease suggested that similar whole blood or plasma concentrations of the two drugs could be achieved if SXZ were given in a priming dose of 3 gm. followed by 1 gm. every 6 hours, and SMP given in a priming dose of 2 gm. followed by 0.5 gm. every 12 hours. (See Figs. 1 and 2). Patients were therefore given appropriate directions according to the medication group to which they were

TABLE I

Sulfonamide Concentrations in Plasma and Urine of Patients

(Mean ± S.E.; mg. per cent)

	Plasma		Urine	
	Free	Total	Free	Total
Sulfisoxazole	4.6 ± 0.6	8.9 ± 1.8	51.3 ± 14.4	106.5 ± 25.6
(No. determinations)	(23)	(12)	(11)	(10)
(No. patients)	(16)	(7)	(8)	(8)
Sulfamethoxypyridazine	14.1 ± 1.0	17.7 ± 2.7	28.2 ± 4.8	83.1 ± 19.3
(No. determinations)	(25)	(11)	(10)	(13)
(No. patients)	(15)	(6)	(6)	(7)

assigned, were given a supply of drug and told to return in one week. At the return (weekly) visits, urinalysis and urine culture were again obtained, blood was drawn for sulfonamide determination, and symptoms noted. For four patients in each treatment group, drug was administered and repeat observations made for only one week; for 7 patients in the SXZ group and 8 patients in the SMP group, two weeks of treatment were employed; the remaining patients (5 in the SXZ group and 3 in the SMP group) received drug for 3–4 weeks.

All sulfonamide concentrations were determined by the Bratton-Marshall procedure (4). In the first half of the study, only free[5] plasma concentrations were measured, whereas in the second half free and total concentrations of drug were determined in both plasma and urine, the urinary determinations being made on casual urine specimens obtained at the time of the weekly clinic visit. The results are summarized in Table I. It would appear, on the basis of the blood and urine sulfonamide concentrations at the time of clinic visits, that the dosage of SXZ and SMP ingested by the patients did result in higher urinary concentrations of SXZ, but that higher plasma levels were achieved with SMP. Because of the pharmacologic characteristics of SXZ and SMP, it is likely that the plasma and urine content of sulfonamide is more variable from hour to hour in the case of SXZ, and that this variability may have been compounded by the necessarily erratic timing of samples (in relation to drug taking) in the clinic situation, and perhaps by a tendency of the outpatients to omit occasional doses of drug (a more important consideration in the case of SXZ).

The therapeutic results were somewhat disappointing with both agents. Urine cultures prior to treatment revealed one or more of the following organisms: *Escherichia coli*, *Aerobacter aerogenes*, *Paracolon bacilli*, *Streptococcus fecalis*, *Klebsiella*, *Proteus vulgaris* or *morgagni*, or *Staphylococcus aureus*. Approximately half of the patients in each group had mixed flora present on the

[5] "Free" is used throughout this paper to mean "non-acetylated".

initial urine examinations. In no patient was the urine rendered bacteria-free by either drug. Urinalysis revealed decrease in pyuria in 82 per cent of patients in the SMP group and in 73 per cent of patients in the SXZ group. There was some improvement in symptomatology in 86 per cent of the SMP group and in 73 per cent of the SXZ group. There was thus no striking difference between treatments, and in the absence of an untreated control group it is impossible to know how much of the decrease in pyuria, urinary frequency, "burning", etc. was attributable to the drugs. Furthermore, while the mean urinary concentration of SMP was considerably lower than that of SXZ, the plasma concentration of SMP was significantly higher, making it difficult to assess the relative importance of drug concentrations in blood and urine. It seemed desirable to perform an animal experiment, where the variables could be more effectively controlled.

B. Animal data

A modification of the procedure of Braude, Shapiro, and Siemienski (5) was used to produce acute pyelonephritis in rats. Male Sprague-Dawley rats weighing 200–300 gm. were used. A strain of *Escherichia coli* isolated from a patient with a urinary tract infection was used as the infecting agent. *In vitro* studies revealed that this organism was equally suppressed by SXZ and SMP in increasing degree by concentrations of drug ranging from 10 to 200 mg. per cent with complete suppression of growth only at concentrations of 150 mg. per cent or higher. The organism was transferred to agar slants seven times over a period of 14 months. One-quarter of a ml. of an 18 hour culture in infusion broth was injected into the tail vein of each rat while the rat was under ether anesthesia. Plate counts on the inocula revealed $4–8 \times 10^8$ organisms per ml.; each rat thus received one to two hundred million organisms in the original inoculum. Immediately after intravenous injection of the *E. coli* organisms, the rat's kidneys were massaged through the intact abdominal wall for 5 minutes. The animals were then put into individual cages and fed Purina chow for 4 days. Of 76 rats injected, 16 (21 per cent) died within 24 hours of injection. The remainder were allocated, 4 days after infection, to 4 groups at random: Group A rats were killed immediately; Group B rats were put on powdered Purina chow containing 1.4 per cent SXZ; Group C rats were placed on powdered Purina chow containing 0.2 per cent SMP; and Group D rats were given only powdered Purina chow. The food was placed in feeding cups, which were weighed and refilled every 2 days for a 10 day period. Fourteen days after infection, the animals in Groups B and C were bled for sulfonamide determinations, and then (along with the rats in Group D) were sacrificed. The kidneys of the animals in all groups (A to D) were examined immediately after sacrifice and graded 0 to IV on the basis of gross lesions (subcapsular and cortical abscesses, medullary streaking, etc.), with 0 being normal and IV being

swollen kidneys with medullary streaking and multiple coalescent abscesses. Then the kidneys were fixed in Zenker's formalin solution, sectioned, and stained with hematoxylin and eosin. The microscopic examination of this material was carried out by Dr. William J. Cathey. Slides were provided to Dr. Cathey identified only by number, and were then graded 0 to IV according to the following scheme:

"The lesions have an appearance similar to that seen in pyelonephritis in humans. They varied from small radial zones of mixed mononuclear and polymorphonuclear cell infiltration in the cortex, to extensive wedge-shaped areas of inflammation extending into the medulla, with purulent exudate in convoluted and collecting tubules, and abscess formation. Pyelitis was usually absent or only mild. Kidneys showing significant abscess formation were listed as Grade IV. Kidneys listed as Grade I showed only a few small cortical lesions. Grades II and III represent more severe degrees of involvement short of abscess formation" (6).

TABLE II

Food and Drug Intake in Infected Rats

(Gms./rat/day)

Days After Infection	Controls (Group D)	Sulfisoxazole Treated (Group B)		Sulfamethoxypyridazine Treated (Group C)	
	Food	Food	Drug*	Food	Drug†
5th and 6th	17.7 ± 4.8‡	16.8 ± 1.3‡	0.235§	18.8 ± 1.8‡	0.038§
7th and 8th	22.8 ± 3.4	20.4 ± 1.7	0.290	22.8 ± 1.4	0.046
13th and 14th	20.6 ± 2.5	23.0 ± 1.4	0.322	25.0 ± 1.7	0.050

* SXZ intake = 1.4 per cent of food intake.
† SMP intake = 0.2 per cent of food intake.
‡ Mean ± standard error of mean.
§ Mean.

TABLE III

Gross Renal Lesions in Infected Rats*

Group	N†	Mean ± Standard Error	Per cent Normal
A (4 day controls)	30	3.33 ± 0.16	0 per cent
B (sulfisoxazole treated)	32	1.25 ± 0.15	18 per cent
C (sulfamethoxypyridazine treated)	30	1.11 ± 0.19	32 per cent
D (14 day controls)	28	2.79 ± 0.21	4 per cent

* Graded as described in text; 0 (no gross abnormality) to IV (swollen kidneys with medullary streaking and massive confluent abscesses).
† N = No. of kidneys (each kidney rated separately).

TABLE IV

Microscopic Renal Lesions in Infected Rats*

Group	N†	Mean ± Standard Error	Per cent Normal
A (4 day controls)	30	2.26 ± 0.21	6 per cent
B (sulfisoxazole treated)	32	1.12 ± 0.13	22 per cent
C (sulfamethoxypyridazine treated)	30	1.13 ± 0.21	32 per cent
D (14 day controls)	28	2.11 ± 0.23	14 per cent

* Pathology as described in text; 0 (no abnormality) to IV (significant abscess formation).
† N = No. of kidneys (each kidney rated separately).

TABLE V

Sulfonamide Concentrations in Infected Rats

(mg. per cent*; Mean ± S.E.)

Group	Plasma	Whole Blood
	Mean ± Standard Error	Mean ± Standard Error
B Sulfisoxazole treated	13.5 ± 0.89	9.7 ± 0.53
C Sulfamethoxypyridazine treated	13.1 ± 0.51	9.6 ± 0.42

* of free drug.

The results are shown in Tables II–V. In Table II it will be seen that the amount of food consumed was similar in drug and control groups. The majority of animals showed some degree of both gross and microscopic abnormality, but the drug-treated groups showed significantly less than the controls (Tables III and IV), although the two drug groups differ remarkably little from each other. The drug groups also showed almost identical whole blood and plasma concentrations of free sulfonamide (see Table V); obviously the urinary concentration of sulfonamide in the SXZ rats, although not actually measured, because of technical difficulties, was considerably higher than in the SMP rats, since the SXZ rats received seven times as much drug in their diet as the SMP rats.

DISCUSSION

A number of investigators (7, 8, 9, 10) seem to agree that sulfamethoxypyridazine is useful in treating urinary tract infections. It is likely that the drug (like other sulfonamides) is variably effective, depending on such factors as the presence of chronic infection, obstructive or calculous uropathy, strain of organism, and the presence of mixed flora in the urinary tract. It has been

a matter of some dispute for years whether urinary or blood concentration of active drug is the important factor in the treatment of urinary infection with sulfonamides (11). The pathology of pyelonephritis suggests that tissue (and therefore plasma) concentrations ought to be more relevant than urinary concentrations (11); whether infections such as cystitis might be more rationally expected to be influenced by urinary concentrations is a frequently voiced but unproven opinion.

Our animal data would seem in keeping with the belief that plasma concentrations are more decisive than urinary concentrations in treating pyelonephritis. It is possible to argue that despite the great disproportion in amount of free SXZ and SMP excreted in the urine, *both* sulfonamides were present in the urine of the infected rats in concentrations above the maximal level required. Such reasoning seems unlikely to us, for two reasons:

1) although it appears that sulfonamides can indeed "cure" experimental pyelonephritis in the rat, the dosage of drugs employed in this experiment cured only a small percentage of the treated rats, so that SXZ and SMP do not appear to have been administered in optimal or supraoptimal dosage;

2) the calculated urinary concentration of SMP[6] was considerably lower than that needed to suppress growth of the infecting organism completely *in vitro*, whereas the urinary concentration of SXZ in the rats should have been sufficient to sterilize the urine; yet both drugs had equal effects. This production of equal therapeutic benefit in the presence of equal blood concentrations and disparate oral intake (and hence urinary concentrations) of the two agents places experimental pyelonephritis in the same category as other non-urinary infections in experimental animals (3).

Our clinical experience with SMP blood concentrations seems explainable by the findings of other workers (8, 12) that there is some cumulation of SMP over a period of days, and that the pilot experiments described in Figures 1 and 2 were probably deceptive as guides to the concentrations achieved in the plasma over a week or more of treatment.

CONCLUSIONS

1) In a controlled clinical trial of sulfamethoxypyridazine and sulfisoxazole, both agents appeared about equally effective in treating a variety of urinary tract infections.

2) In experimentally-produced pyelonephritis in rats, both drugs, when given orally in doses yielding unequal urinary but equal plasma concentrations of free sulfonamide, were equally effective in decreasing gross and microscopic renal lesions.

3) These data seem to favor the hypothesis that plasma (and tissue) con-

[6] Data on the rat in similar experiments (13) revealed free SMP concentrations in the urine of 16 to 90 mg. per cent.

centrations of sulfonamide are more important determinants of efficacy in treating certain types of urinary tract infection than are urine concentrations.

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HEMORRHAGIC ENCEPHALOPATHY IN CHICKEN EMBRYOS INFECTED WITH INFLUENZA VIRUS

I. Factors Influencing the Development of Hemorrhages[1]

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INTRODUCTION

Newly isolated strains of influenza virus multiply in the amnion and respiratory epithelium of chicken embryos (1, 2, 3) and can easily be adapted to grow in the allantoic sac or on the chorioallantoic membrane (4, 5). Allantoic or chorioallantoic infections usually remain localized (1, 5), but death with generalized hyperemia of tissues and multiple small hemorrhages often occurs after amniotic infection in embryos less than 10 days of age (4, 6).

Three variants of influenza A virus regularly invade the embryo from the chorionic surface of the chorioallantoic membrane, causing extensive hemorrhagic lesions and death. These "invasive" forms, Melbourne-Egg (Mel-Egg) (7), WS-Egg (WSE) (8) and "neurotropic" WS (NWS) (9), emerged during serial chorioallantoic passage of the parent strains. NWS virus caused extensive brain hemorrhages after 14 consecutive passages, whereas 63 passages of Mel-Egg were required to produce the same effect. The pathogenicity of the Mel-Egg virus was further enhanced by the 71st passage, at which time infected embryos showed diffuse hemorrhages in skin and muscle as well as brain.

The experiments reported in this series of papers were designed to study the pathogenesis of hemorrhagic encephalopathy in chicken embryos and to test the hypothesis of Burnet and Fraser (10) that "invasive" strains of influenza virus infect capillary endothelium of the brain. The present report demonstrates that development of vascular lesions depends on the age of the embryo, route of infection, and multiplication potential of the virus. The pathology of the cerebrovascular lesions is described in the second paper (11).

Materials and Methods

Viruses. Allantoic fluid preparations of the 33rd to 36th egg passage of the Stuart-Harris (9) "neurotropic" variant of WS virus (NWS) were used in most experiments. The non-neurotropic WS and PR8 strains of influenza A virus and the Lee Strain of influenza B virus, each of which had undergone more than 100 allantoic passages, were also tested for their capacity to cause hemorrhagic encephalopathy. All studies were carried out with infected allantoic fluids harvested from 12 day old chicken embryos which had been inoculated intra-

[1] These studies were supported by a Grant from the U. S. Public Health Service and a Contract with the U. S. Army Chemical Corps, Fort Detrick, Frederick, Md.

allantoically 48 hours previously with 0.1 ml. of a 10^{-3} dilution of seed virus. Hemagglutinin and infectivity titers of each standard virus suspension were determined prior to storage at $-60°$ C.

Methods of Infecting Embryos. Intravenous, allantoic and chorio-allantoic routes of inoculation were compared. Intravenous injection was made in a large allantoic vein after removing a 0.5 x 1.0 cm. portion of egg shell. A drop of sterile mineral oil was placed on the exposed shell membrane and, with the aid of transillumination, 0.1 ml. of material was injected through a 27-gauge needle introduced into the vein in the direction of blood flow. The window in the shell was sealed with a mixture of melted paraffin and beeswax.

Allantoic fluid suspensions of virus were diluted in broth-saline solution containing 0.25 gm. of neopeptone, 0.8 gm. of sodium chloride, 0.037 gm. of glucose, and 0.5 gm. of beef infusion in each 100 ml. Intravenous injection of normal allantoic fluid (NAF) is lethal for most embryos (12), but fewer than 10 per cent die if the inoculum is diluted tenfold or greater with broth-saline solution.

Chorioallantoic and allantoic inoculations were made by standard techniques (12).

Scoring Brain Lesions. Hemorrhages were graded 0, 1+, 2+ or 3+, according to their extent and severity. The brain lesion score for each group of embryos was expressed as the sum of the individual lesions divided by the number of embryos times 3 (greatest possible score), and converted to percentage:

$$\text{Brain lesion score (\%)} = \frac{\text{Sum of individual hemorrhages}}{3 \times \text{No. of embryos}} \times 100$$

Thus, a brain lesion score of 100 per cent indicates 3+ hemorrhages in all embryos of a group.

Methods of Obtaining Blood, Brain and Liver for Infectivity Determinations. Blood was withdrawn from an allantoic vein at a site distant from the previous intravenous inoculation. Approximately 0.1–0.3 ml. of blood could easily be obtained without contamination by allantoic fluid. The surface of each embryo was then washed with 500 ml. of sterile normal salt solution to minimize contamination of brain and liver with virus present in extra-embryonic fluids. The liver was removed through an incision in the ventral abdominal wall. Normal brains and those with small hemorrhages were removed intact after reflecting the skin and calvarium with separate sterile instruments. The liquid consistency of markedly hemorrhagic brains necessitated aspiration of the cranial contents with a 19-gauge needle introduced through an area of skull seared with a hot iron.

Infectivity Titrations. Individual brains or livers were homogenized in TenBroeck tissue grinders with sufficient broth-saline solution to make a 10 per cent suspension. Tissue suspensions, blood or extra-embryonic fluids were diluted with broth-saline solution in tenfold steps, and 0.1 ml. aliquots of each dilution were injected into the allantoic cavities of 6 eggs 10 or 11 days of age. After an additional 48 hours of incubation at 38° C. eggs were chilled and allantoic fluids tested individually for their capacity to agglutinate an equal volume of one per cent chicken erythrocytes. The 50 per cent egg-infective dose (EID_{50}) was determined by the method of Reed and Muench (13).

EXPERIMENTAL

Development of Cerebral Hemorrhages in Embryos Infected with NWS Virus. Fourteen day old chicken embryos inoculated intravenously with small doses of NWS virus developed punctate hemorrhages in the cerebral and cerebellar cortex 24–30 hours after infection. Thereafter, hemorrhages increased in number and severity, became confluent, and caused the cranial contents to bulge

FIG. 1. Chicken embryos, 16 days old. *Left*, normal embryo. *Right*, infected embryo 64 hours after NWS virus was inoculated intravenously.

FIG. 2. Sagittal sections of chicken embryo heads. *Left*, normal embryos. *Right*, hemorrhagic brain lesions resulting from intravenous infection with NWS virus.

superiorly (Figs. 1 and 2). In several experiments 85–100 per cent of the embryos were dead with marked cerebral hemorrhages 48–72 hours after infection.

Minute hemorrhages occurred occasionally in skin and muscle of 14 day old embryos infected with NWS virus, but the most intense hemorrhages were always observed in the brain.

Influence of Age and Route of Inoculation on Mortality and Development of Cerebral Hemorrhages.

Embryos 10, 12, 14 and 16 days of age were infected with NWS virus by the chorioallantoic, allantoic or intravenous routes. The LD_{50} was determined for each group of 64 or 80 embryos injected with aliquots of decimal dilutions of a standard virus suspension. Deaths occurring within 24 hours were considered nonspecific and the experiment was terminated in 4 days.

No significant difference was observed in the LD_{50} of NWS virus inoculated by the allantoic, chorioallantoic or intravenous routes in 10 and 12 day old embryos, but with increasing age, embryos became more resistant to allantoic or chorioallantoic infection (Table I). In 14 and 16 day old embryos, 100 to 100,000 times more virus was required to initiate a lethal allantoic or chorioallantoic infection than in 10 and 12 day old embryos. However, no increased resistance was noted in older embryos infected by the intravenous route; at 16 days of age embryos were as susceptible to the lethal effect of intravenously administered virus as they were at 10 days of age.

Newly hatched chicks inoculated intravenously with 20,000 EID_{50} of NWS virus showed no signs of illness during a period of 6 days.

The influence of age of the embryo and route of inoculation on the occurrence of brain hemorrhages was studied in 10, 12, 14 and 16 day old embryos infected with serial tenfold dilutions of NWS virus by the allantoic, chorioallantoic or intravenous routes. The presence or absence of hemorrhages was noted in embryos dead of infection and those that survived for 96 hours, when the experiments were terminated. The brain lesion scores are based on groups of 64 or 80 embryos.

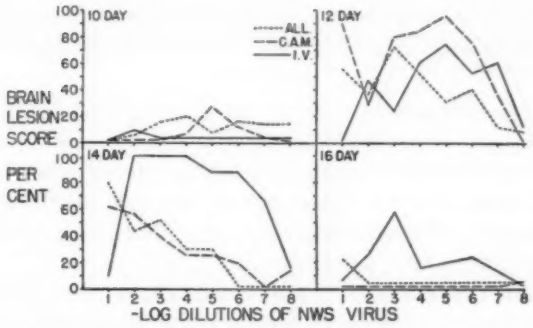
Cerebral hemorrhages occurred infrequently in 10 day old embryos regardless of the route of inoculation or the infecting dose (Text-fig. 1). Allantoic or chorioallantoic injection of large doses of NWS virus had little effect in 16 day old embryos, and intravenous injection caused only a moderate number of brain lesions. In contrast, brain hemorrhages were noted in 12 day old embryos infected with high dilutions of NWS virus by any route of inoculation.

Intense hemorrhages occurred most consistently when infection was initiated by the intravenous route in 14 day old embryos; as little as 2 EID_{50} of NWS virus resulted in a brain lesion score of 90 per cent.

TABLE I

LD_{50} of NWS Virus Injected by Various Routes in Chicken Embryos of Different Ages

Route of Inoculation	Age of Embryos When Inoculated, *days*			
	10	12	14	16
Allantoic	$10^{-7.6}$	$10^{-7.4}$	$10^{-4.8}$	$10^{-3.2}$
Chorioallantoic	$10^{-7.5}$	$10^{-7.2}$	$10^{-5.2}$	$10^{-2.8}$
Intravenous	$10^{-7.2}$	$10^{-7.7}$	$10^{-7.6}$	$10^{-7.2}$



TEXT-FIGURE 1. Degree of brain hemorrhage in 10, 12, 14 and 16 day old chicken embryos infected with NWS virus by the allantoic (All.), chorioallantoic (C.A.M.), or intravenous (I.V.) routes.

TABLE II

Hemagglutinin in Extra-embryonic Fluids of Chicken Embryos Infected Intravenously with 40 EID_{50} of NWS Virus

Experiment No.	Embryos* Tested	Brain Lesion Score	Hemagglutinin Present in:			
			Allantoic fluid alone	Amniotic fluid alone	Allantoic and Amniotic fluids	Neither
		per cent				
1	31	96	1	11	15	4
2	12	100	3	1	3	5

* 14 days old when infected.

High concentrations of virus often caused less marked cerebral lesions than low concentrations. This paradoxical effect was particularly evident in 12, 14 and 16 day old embryos inoculated intravenously. Despite the failure of concentrated virus to produce marked brain hemorrhages, embryos invariably died 48–72 hours after infection.

Infection of the Allantois and Amnion after Intravenous Injection of NWS Virus. Burnet and Fraser (10) were unable to detect viral hemagglutinin in allantoic and amniotic fluids of chicken embryos infected by the intravenous route with a small dose of NWS virus.

Embryos 14 days of age were inoculated intravenously with 40 EID_{50} (equivalent to 400 intravenous LD_{50}) of NWS virus. Allantoic and amniotic fluids from embryos developing marked hemorrhages 48–72 hours later were tested for the presence of agglutinins for chicken erythrocytes.

As shown in Table II, 79 per cent of 43 embryos infected with 40 EID_{50} of NWS virus developed hemagglutinin in allantoic or amniotic fluid, or both. Marked cerebral hemorrhages were observed in all embryos, irrespective of the presence of hemagglutinin in extra-embryonic fluids.

Those allantoic and amniotic fluids giving negative hemagglutinin reactions ($< 1:2$), as well as brain tissue from the same intravenously infected embryos, were tested individually for their content of infectious virus.

Table III demonstrates that significant amounts of infectious virus were present in the extra-embryonic fluids despite the absence of hemagglutinin. However, the titers of virus in brain far exceeded those of allantoic or amniotic fluids of these same embryos. It is apparent, therefore, that NWS virus could invade the allantois and amnion after intravenous infection, but its subsequent multiplication at these sites was limited.

Hemagglutinin Production vs. Mortality as Criteria of Infection with NWS Virus. As WS virus underwent adaptation to the "neurotropic" form (NWS) by serial passage in chicken embryos and mice (9), its infectivity for allantoic cells of the chicken embryo diminished. Despite 36 subsequent allantoic passages, at least 10 times more NWS virus than WS virus is required to produce detectable hemagglutinin after intra-allantoic infection.

Two allantoic fluid preparations of NWS virus were titrated by the allantoic route in 10 or 12 day old embryos and, in two parallel experiments, by the intravenous route in 12 or 14 day old embryos. Six to 10 embryos were inoculated at each dilution. Embryos injected intra-allantoically were considered to be infected if hemagglutinin was detected in allantoic fluid after incubation for 48 hours. The criterion of infection in embryos injected intravenously was death within 96 hours.

Table IV demonstrates that the intravenous LD_{50} of NWS virus was 10 to 50 times higher than the allantoic EID_{50}. Therefore, the classical allantoic method for titrating infectivity of influenza viruses was capable of detecting fewer than a tenth of the infectious units of NWS virus contained in infected allantoic fluid. It is also noteworthy that NWS virus titers of $10^{-7.7}$ and $10^{-8.0}$ determined by the sensitive intravenous method compared favorably with titers of the parent WS strain determined by allantoic infection. It appears,

TABLE III

Comparison of Virus Content of Brain and Extra-Embryonic Fluids of Chicken Embryos Infected Intravenously with NWS Virus

Experiment	Embryo No.	I.V. Dose	Degree of Brain Lesions	Virus Titer (Log EID_{50})		
				Allantoic fluid*	Amniotic fluid*	Brain
		EID_{50}				
1	1	40	3+	4.3	4.0	6.6
	2	40	3+	3.3	4.3	7.1
2	1	20	3+	4.1	—	6.5
	2	20	3+	5.1	—	6.6
	3	20	3+	5.3	—	6.1

* Hemagglutinin not detected in extra-embryonic fluids.

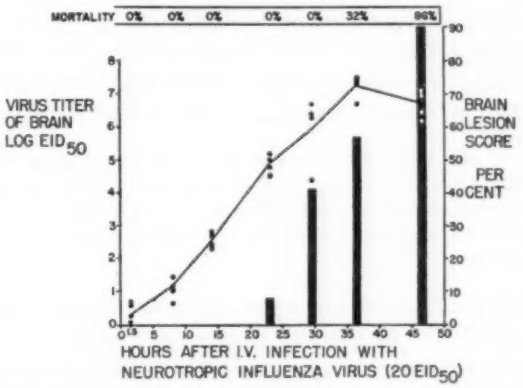
TABLE IV

Comparative Susceptibility of Chicken Embryos to Intravenous and Allantoic Infection with NWS Virus

Preparation of Allantoic Fluid	Titer of Virus	
	Allantoic infection	Intravenous infection
	EID_{50}*	LD_{50}†
No. 1	$10^{-6.6}$	$10^{-7.7}$
No. 2	$10^{-6.3}$	$10^{-8.0}$

* Based on the presence of hemagglutinin 48 hours after allantoic inoculation of 10 and 12 day old chicken embryos.

† Based on death within 96 hours after intravenous inoculation of 12 and 14 day old chicken embryos.


MORTALITY 0% 0% 0% 0% 0% 32% 96%
VIRUS TITER OF BRAIN LOG EID50
BRAIN LESION SCORE PER CENT
HOURS AFTER I.V. INFECTION WITH NEUROTROPIC INFLUENZA VIRUS (20 EID50)

TEXT-FIGURE 2. Growth curve of NWS virus in brain and its relationship to cerebral hemorrhages and death. Note that the development of brain lesions lagged behind virus multiplication.

therefore, that allantoic cells are fully capable of supporting the multiplication of NWS virus, but the resulting progeny have a limited capacity for initiating allantoic infection in a new host.

Relation of NWS Virus Multiplication to Development of Brain Hemorrhages. The occurrence of cerebral hemorrhages and death was directly proportional to the rapid multiplication of NWS virus in brain.

A group of chicken embryos 14 days of age was inoculated intravenously with 20 EID_{50} of NWS virus (equivalent to 1000 i.v. LD_{50} of this preparation). Eight embryos were selected at random 1½, 8, 14, 23, 36 and 46 hours after infection to determine the brain lesion score, and at each time interval the brains of 4 of these embryos were titrated individually by allantoic inoculation of eggs. The cumulative mortality rate was calculated concurrently.

The virus content of individual brains harvested at each interval was strikingly similar, evidence of a uniform rate of virus proliferation (Text-fig. 2).

Little virus was detected in the brain 1½ hours after intravenous inoculation, but the titer increased rapidly thereafter, reaching a maximum level of 10^{9} EID_{50} at 36 hours.

Significant gross lesions were not observed until the titer of virus in brain was 10^{-5}, when small hemorrhages developed. The brain lesion score increased rapidly from the 30th hour after infection, and most embryos showed extensive cerebral hemorrhages 36 to 46 hours after infection. When the brain lesion score was maximum (90 per cent) 46 hours after infection, the titer of virus in brain had decreased from the peak achieved at 36 hours.

Rarely, a moribund embryo was observed with diffuse cerebral hemorrhages, but extensive lesions eventually resulted in death. All infected embryos were alive 30 hours after infection when the titer of virus in the brain was 10^{-6}; thereafter, the mortality rate increased rapidly to 86 per cent at 46 hours.

Comparative Growth Curves of NWS Virus in Brain and Liver. Although cerebral hemorrhages were the only lesions of internal organs visible by gross inspection, the titer of virus increased as rapidly in liver as in brain.

A group of 14 day old chicken embryos was inoculated intravenously with 20 EID_{50} of NWS virus (equivalent to 1000 i.v. LD_{50}). Four embryos were selected at random 1, 3, 6, 9, 12, 18 and 24 hours after inoculation and the titer of virus in allantoic fluid, blood, brain and liver of each embryo was determined individually by subinoculation into the allantoic sac of 10–12 day old embryos.

Infectious virus was first detected in liver and brain at 6 hours and multiplied rapidly to a titer of 10^{-6} 24 hours after infection (Table V). No significant difference was observed in virus titers of brain and liver at any interval after infection.

Viremia. Table V also demonstrates that virus appeared in the blood 12–18 hours after intravenous injection of 20 EID_{50} of NWS virus. It should also be noted that the virus content of liver and brain exceeded that of blood at any given time. However, viremia preceded detectable allantoic infection by at least 6 hours, indicating that virus invasion of the allantois was secondary to blood stream infection.

As noted in Table VI, virus invariably was present in blood 24 hours after intravenous infection of 13 additional embryos, but the infectivity titers ranged from $10^{-2.6}$ to $10^{-6.5}$. Spontaneous agglutination of erythrocytes occasionally occurred when venous blood of infected embryos was suspended in normal saline solution. However, there was no correlation between spontaneous agglutination of erythrocytes and the concentration of infectious virus of the blood.

Effect of Other Influenza Viruses Injected Intravenously. Burnet and Fraser (10) reported that a non-invasive Mel strain of influenza A virus inoculated intravenously in large amounts produced hemorrhagic brain lesions.

TABLE V

Rate of Virus Multiplication in Chicken Embryos Infected Intravenously with 20 EID_{50} of NWS Virus

Embryo No.	Hours After Infection	Log EID_{50} of NWS Virus			
		Allantoic fluid	Blood	Liver	Brain
1	1	<1.0	<1.0	<1.0	<1.0
2		<1.0	<1.0	<1.0	<1.0
3		<1.0	<1.0	<1.0	<1.0
4		<1.0	<1.0	<1.0	<1.0
5	3	<1.0	<1.0	<1.0	<1.0
6		<1.0	<1.0	<1.0	<1.0
7		<1.0	<1.0	<1.0	<1.0
8		<1.0	<1.0	<1.0	<1.0
9	6	<1.0	<1.0	1.3	1.6
10		<1.0	<1.0	2.1	1.7
11		<1.0	<1.0	2.1	1.7
12		<1.0	<1.0	2.1	1.2
13	9	<1.0	<1.0	<1.0	2.0
14		<1.0	<1.0	2.1	2.2
15		<1.0	<1.0	1.2	1.7
16		<1.0	<1.0	1.6	1.5
17	12	<1.0	1.6	3.4	3.4
18		<1.0	<1.0	3.5	2.7
19		<1.0	<1.0	2.6	2.6
20		<1.0	<1.0	3.1	3.5
21	18	<1.0	1.6	4.5	4.8
22		<1.0	2.5	4.2	4.3
23		<1.0	2.7	4.2	4.0
24		<1.0	2.1	5.3	5.5
25	24	2.2	3.0	6.1	5.8
26		1.2	3.7	6.5	6.2
27		<1.0	3.2	4.3	5.2
28		<1.0	4.4	6.2	6.9

The effect of intravenous inoculation of the WS, PR8, and Lee strains of influenza virus in 14 day old chicken embryos was studied for comparison with the "invasive" NWS strain. The Lee strain did not produce hemorrhagic brain lesions or a significant number of deaths after intravenous inoculation (Table VII). PR8 and WS viruses were lethal for chicken embryos infected intravenously in doses of 5,500 or 220 EID_{50}, respectively; cerebral hemorrhages occurred sporadically and these were only occasionally as marked as those with NWS virus.

The capacity of WS virus to multiply in brain was relatively limited. Virus titers in brains of 4 embryos 48–72 hours after intravenous injection of 31,000 EID_{50} of WS virus ranged from $10^{-4.7}$ to $10^{-5.4}$, compared with titers of $10^{-6.7}$ to $10^{-7.5}$ 36 hours after injection of 20 EID_{50} of NWS virus.

TABLE VI

Viremia in Chicken Embryos 24 Hours After Intravenous Inoculation of 20 EID_{50} of NWS Virus

Embryo No.	Presence of Virus in Blood	
	Hemagglutinin*	Log EID_{50}
1	+	4.2
2	0	2.6
3	0	3.3
4	+	5.0
5	0	6.5
6	0	3.4
7	0	3.1
8	0	6.4
9	0	4.6
10	0	3.7
11	0	5.2
12	0	4.3
13	0	4.0

* Spontaneous agglutininination of 1 drop of blood suspended in 1 ml. of normal saline solution.

TABLE VII

Effect of Intravenous Inoculation of PR8, WS, and Lee Strains of Influenza Virus in 14 Day Old Chicken Embryos

Strain of Virus	Dose Inoculated	Deaths*	Brain Lesion Score
	Log EID_{50}		*per cent*
PR8	5.74	8/8	13
	4.74	9/9	0
	3.74	4/8	0
	2.74	0/9	0
WS	6.11	6/6	0
	5.11	10/10	40
	4.11	10/10	23
	3.11	8/9	0
	2.11	3/9	0
	1.11	1/9	0
Lee	6.61	3/6	0
	5.61	1/6	0
	4.61	1/6	0
	3.61	0/6	0

* Observation period: 5 days.

DISCUSSION

Previous studies (14) have shown that NWS virus causes generalized infection in infant mice injected by intraperitoneal, intranasal or intramuscular routes. Viremia develops with small infecting doses and the virus is disseminated to and multiplies in all tissues. Susceptibility to infection diminishes with increasing age and newly weaned mice are resistant to parenteral injection of

virus. This "invasive" property of NWS virus in immature hosts had previously been demonstrated in chicken embryos. Virus gains access to the circulation from the chorioallantoic membrane and multiplies extensively in brain with the production of marked cerebral hemorrhages (9). However, Burnet and Fraser (10) noted that cerebral hemorrhages occur less frequently in 12 day old embryos inoculated with NWS virus by the allantoic route. These differences in the incidence of hemorrhagic encephalopathy after chorionic and allantoic infection were also observed in the present studies, but to a lesser extent and only in 12 day old embryos. At 14 days of age, embryos were moderately resistant to both chorionic and allantoic infection and by 16 days of age even large doses of NWS virus injected by these routes failed to produce cerebral hemorrhages.

In contrast, embryos 10, 12, 14 and 16 days of age were equally susceptible to the lethal action of NWS virus injected directly into the blood stream. The incidence of cerebral hemorrhages after intravenous infection of 14 day old embryos was correspondingly high (50% brain lesion score: LD_{50} ratio of $10^{7.0}$:$10^{7.6}$), whereas embryos 10, 12 and 16 days of age frequently died without gross cerebral hemorrhages. Notwithstanding, it appears that a partial barrier to viral invasion of the embryo from chorionic and allantoic sites of infection develops with increasing age.

The existence of allantoic and amniotic barriers to infection in 14 day old embryos was also demonstrated by studies of virus dissemination after intravenous injection. In the early stage of infection NWS virus could not be detected in allantoic fluid although present in the blood of the same embryos. Virus eventually appeared in extra-embryonic fluids, but at titers lower than anticipated and often in amounts insufficient to be detected by hemagglutination. To some extent these abortive infections of allantois and amnion may be a function of intrinsically limited growth potential of NWS virus at these sites. With the "invasive" Mel-Egg strain, Burnet and Bull (15) found that the EID_{50}, based on appearance of hemagglutinin after allantoic inoculation, was often 100 times less than the LD_{50} as determined by intravenous inoculation of virus. This discrepancy in methods for assaying infectivity also applies to NWS virus (10). In our experiments determination of the EID_{50} by the classical method of allantoic subinoculation gave disproportionately low titers compared to the LD_{50} after intravenous infection, the EID_{50}:LD_{50} ratios ranging from 1:10 to 1:50.

The occurrence of cerebral hemorrhages after intravenous injection of NWS virus was directly related to the localization and multiplication of virus in brain tissue. Even with small infecting doses (20 EID_{50}) the virus proliferated at a rapid and uniform rate reaching maximum levels of infectivity in 36 hours. Brain lesions also developed in a regular sequence. Hemorrhages were first

noted at 24 hours when the virus titers were approximately 10^{-5}, gradually increased in extent and severity thereafter, but were of maximal intensity only after infectivity titers were beginning to decline. The "non-invasive" WS virus had a limited capacity to multiply in the chicken embryo after intravenous injection. The titers of WS virus in the brain 36 hours after infection were 100–1000 times less than titers in embryos infected with NWS virus. Hemorrhagic encephalopathy, regularly observed after small doses of NWS virus, occurred only sporadically in embryos inoculated intravenously with the WS strain. However, WS virus infection frequently resulted in death from other causes, a finding previously noted (10) with the "non-invasive" Mel strain. A similar correlation between multiplication potential and pathogenicity has been noted in influenza virus infections of mouse lung. Mouse adapted strains multiply rapidly to high titer and cause extensive pulmonary lesions, although the development of pneumonitis invariably lags behind virus proliferation (16). Large amounts of unadapted viruses are required to produce comparable pulmonary lesions, and virus multiplication is correspondingly limited (17).

These data do not completely elucidate the pathogenesis of hemorrhagic encepholopathy, but certain conclusions seem warranted. The uniform susceptibility to intravenous infection of embryos of all ages indicates that the encephalopathogenic and lethal actions of NWS virus are dependent to some extent upon its gaining entrance to the blood stream. Significant amounts of virus appeared in the liver and brain between 3 and 6 hours after intravenous injection of a scant 20 EID_{50}, suggesting that virus is immediately transported to highly susceptible cells. Within a further 6 to 12 hours virus multiplied rapidly in the tissues and re-entered the circulation. The most plausible explanation for this sequence of events is that NWS virus infection takes place within the vascular system. The circulating blood cells can be excluded as primary sites of virus multiplication because the tissues invariably contained more infectious virus than the blood. It seems most likely, therefore, that the initial sites of virus localization were in endothelial cells, which would account for the short eclipse phase and the subsequent rapid increase in virus content of the tissues. Presumably, cross infection of endothelial cells also occurred by way of the blood stream when virus was released from infected endothelial cells, although viremia could not be detected until the levels of virus in the tissues exceeded 10^4 EID_{50}. Additional evidence in support of an endothelial site of virus proliferation is presented in the following paper on evolution of vascular lesions in the brains of infected embryos.

It should be noted that NWS virus multiplication in liver paralleled that in brain, evidence for equivalent susceptibility to infection of vascular endothelium of different tissues. No explanation is apparent, therefore, for the occurrence of gross cerebral hemorrhages almost entirely in brain tissue. How-

ever, the viruses of Newcastle disease (18), Rous sarcoma (19), pseudorabies (20), and equine encephalomyelitis (21, 22) also produce hemorrhagic diseases in chicken embryos with lesions predominately in brain. The influence of local anatomical factors rather than propensity of viruses to affect cerebral blood vessels is emphasized by the fact that bacterial endotoxin (23) and certain heavy metals (24) also cause hemorrhagic encepholapathy in chicken embryos by damaging cerebral endothelium.

There appear to be three possible mechanisms by which influenza virus can cause hemorrhagic encephalopathy. The first of these is that virus accumulating in the tissues may eventually reach concentrations sufficient to provoke a toxic reaction, similar to the hemorrhages produced in mice by massive doses of non-infectious virus (25, 26). Secondly, it is possible that cerebral hemorrhages are the result of multiple thrombi formed by erythrocytes agglutinated by circulating virus (15). However, this explanation seems unlikely because no correlation was found between the degree of viremia and spontaneous agglutination of erythrocytes in venous blood removed from embryos with cerebral hemorrhages. Finally, virus multiplying within endothelial cells may injure them directly and cause disruption of vessel walls. The data presented here and in the following paper (11) appear to support the last hypothesis.

SUMMARY

The pathogenesis of hemorrhagic encephalopathy was studied in chicken embryos infected with the "neurotropic" WS strain of influenza A virus. Susceptibility to the lethal and hemorrhagic effects of the virus was dependent upon the age of the embryo and the route of inoculation. Small infecting doses caused death of 10 day old embryos, usually without cerebral hemorrhages, regardless of the route of inoculation. Resistance to allantoic and chorioallantoic infection developed with increasing age. However, marked cerebral hemorrhages occurred in 14 day old embryos infected intravenously with as little as 2 EID_{50} of NWS virus. No significant differences were noted in susceptibility to the lethal action of NWS virus injected intravenously in 10–16 day old embryos. In fact, the incidence of death after intravenous infection was a more sensitive method for detection of NWS virus than the classical method of titration based on the presence of hemagglutinin after allantoic infection.

The WS, PR8 and Lee strains of influenza virus were limited in their capacity to infect chicken embryos by the intravenous route and rarely caused brain lesions.

The development of cerebral hemorrhages was correlated with the multiplication of NWS virus in brain. Following intravenous injection of 20 EID_{50} the titers rose rapidly to a level of 10^{-6} in 24 hours. Gross hemorrhagic lesions were

first noted at this time but were of maximum intensity only after the virus titers had begun to decline. Simultaneous titrations of virus in tissues, blood and allantoic fluid revealed that virus multiplied equally well in brain and liver, but could be detected in venous blood only after infection was well established. Abortive infection of allantoic cells occurred secondary to viremia.

These studies appear to support the hypothesis that hemorrhagic encephalopathy is caused by localization and multiplication of NWS virus in vascular endothelium.

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HEMORRHAGIC ENCEPHALOPATHY IN CHICKEN EMBRYOS INFECTED WITH INFLUENZA VIRUS

II. Pathology[1]

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INTRODUCTION

Hemorrhagic brain lesions are the most prominent gross pathological findings in chicken embryos infected with certain strains of influenza virus (1–3). The preceding paper (4) described the evolution of these lesions and their relation to virus multiplication in the brain. Small cerebral hemorrhages were first noted 24 hours after intravenous infection when the virus content of brain reached a level of 10^5 EID_{50}. Within a further 12 to 22 hours hemorrhages in the brain became confluent and massive, and the embryos died.

The neuropathology of influenzal hemorrhagic encephalopathy is described in the present report. The earliest lesions noted were diffuse damage to capillaries in the absence of specific neuronal lesions or inflammation, suggesting that the "neurotropism" of NWS influenza virus in the chicken embryo is primarily an effect on endothelium rather than on nerve cells.

Materials and Methods

Virus. A single allantoic fluid preparation of the neurotropic variant (NWS) of the WS strain of influenza A virus was used. The seed inoculum was stored at −60° C. and diluted with broth-saline solution to the desired concentration immediately prior to use.

Infection of Embryos. Fourteen day old chicken embryos were injected intravenously with 0.1 ml. of a suspension containing 1000 intravenous LD_{50} of NWS virus. Control embryos were injected intravenously with 0.1 ml. of broth-saline solution. Both groups were incubated at 38° C. and candled frequently to determine the incidence of death. Four embryos of each group were selected at random for pathologic study at intervals after injection. The virus content of the brain of four additional embryos in the virus-infected group was determined at the same time intervals. Methods of scoring brain lesions, removing brains and determining egg infectivity (EID_{50}) were described in the preceding paper (4).

Preparation of Brains for Histologic Study. Embryos were killed by injecting 1–2 ml. of 10 per cent formalin into an allantoic vein. Further fixation of brain was achieved by perfusing the heart with an additional 1–2 ml. of formalin. Small incisions were made in the skull to assure effective penetration of fixative and the entire head was then placed in 10 per cent formalin at 4° C. for 24–48 hours. After this preliminary fixation, the brain was carefully removed from the cranial cavity and immersed in formalin for two weeks, prior to embedding in celloidin. Sections were cut serially at 20 to 30 microns and stained with hematoxylin and eosin, gallocyanin and Van Gieson's stain.

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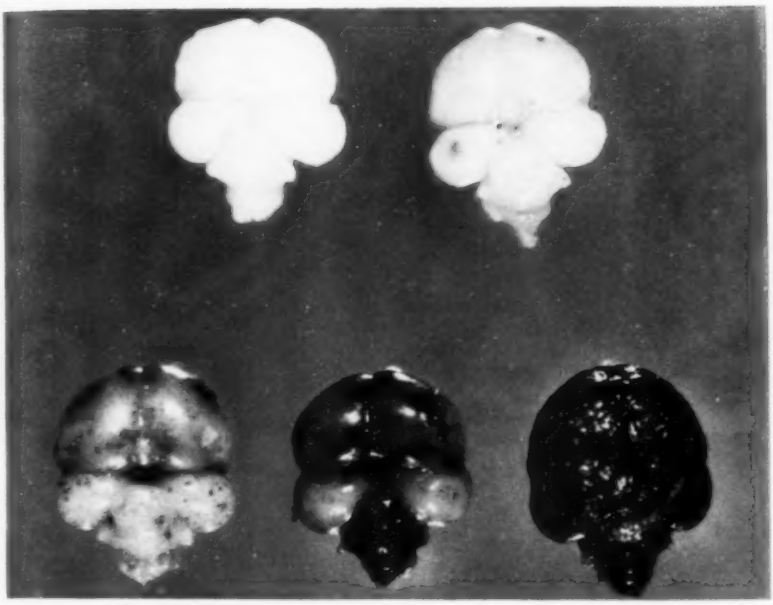

FIG. 1. (Left to right. Top row: A, B. Bottom row: C, D, E) Brains from chicken embryos 1, 24, 30, 36 and 46 hours after infection intravenously with 1000 i.v. LD_{50} of NWS virus.

RESULTS

Gross Pathology. The appearance of chicken embryo brains at intervals after intravenous infection with NWS virus is shown in Figure 1. Twenty-four to 30 hours after infection, punctate hemorrhages, 1 to 3 mm. in diameter, appeared in cerebral and cerebellar cortex, Figure 1 (B, C). Thereafter, these increased progressively in number and size. The punctate hemorrhages became confluent after 36 hours, resulting in massive hemorrhagic softening and edema of brain, Figure 1 (D, E). Hemorrhages sometimes ruptured through the meninges and membranous skull to form subcutaneous hematomas. Volumes and weights of brains with advanced lesions were frequently doubled.

The correlation between degree of gross brain hemorrhage, titer of virus in brain and mortality is shown in Table I. Single punctate hemorrhages were occasionally noted 24 hours after infection but extensive lesions occurred only when the virus titer exceeded 10^6 EID_{50}.

Hemorrhagic brain lesions were the most striking manifestations of NWS virus infection in chicken embryos. Although careful examination of the entire embryo was not performed routinely, the only other lesions noted were occasional small hemorrhages in feather follicles and muscle.

Microscopic Pathology. No lesions were noted in brains of embryos infected

TABLE I

Correlation of Brain Hemorrhage, Titer of Virus in Brain and Mortality in Chicken Embryos Infected Intravenously with 1000 LD_{50} of NWS Virus

Time After Injection	Embryos Examined	Type of Brain Hemorrhage					Mortality	Titer* of Virus in Brain
		None	Single punctate	Multiple punctate	Large confluent	Massive		
Hours							*per cent*	EID_{50}
1	4	4					0	$10^{0.5}$
8	4	4					0	$10^{1.1}$
14	4	4					0	$10^{2.6}$
24	4	3	1				0	$10^{4.9}$
30	4		3	1			0	$10^{6.0}$
36	6				3	3	32	$10^{7.2}$
46	3	1			1	1	86	$10^{6.7}$

* Each value represents the mean titer of 4 embryo brains.

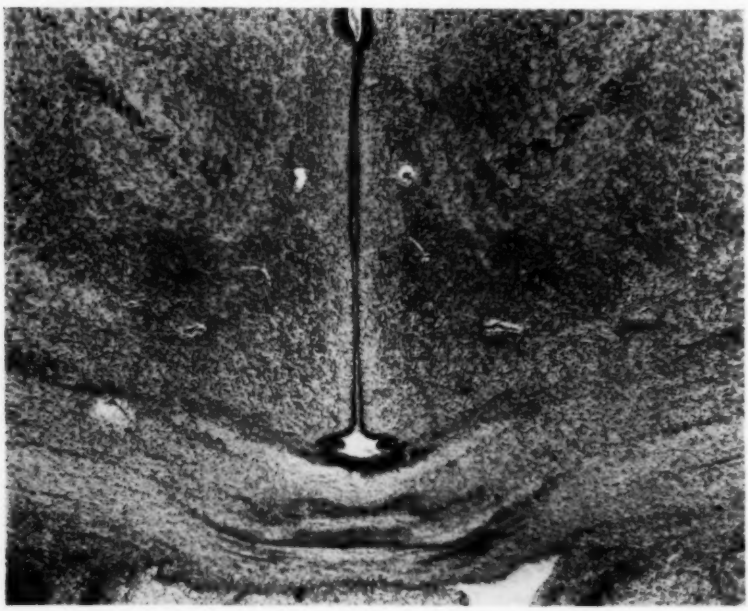

FIG. 2. Section through third ventricle and thalamus 8 hours after infection showing normal vascular pattern (× 50).

intravenously with NWS virus 1 or 8 hours previously (Figs. 2 and 3). Minimal dilatation and engorgement of capillaries and veins were evident at 14 hours, and became marked at 24–30 hours after infection (Figs. 4 and 5). These vascular changes appeared first in the white matter of the cerebral and cerebel-

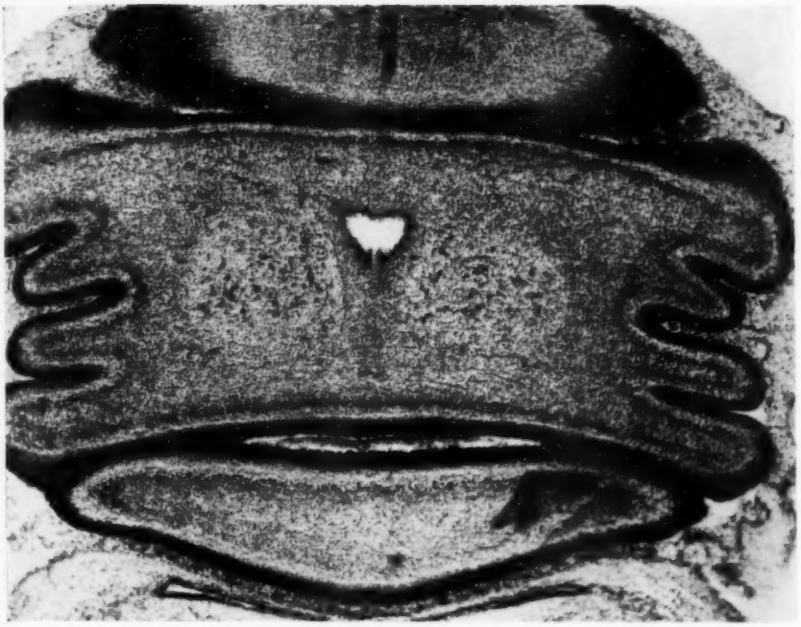

FIG. 3. Section through cerebellum 8 hours after infection showing normal vascular pattern (× 50).

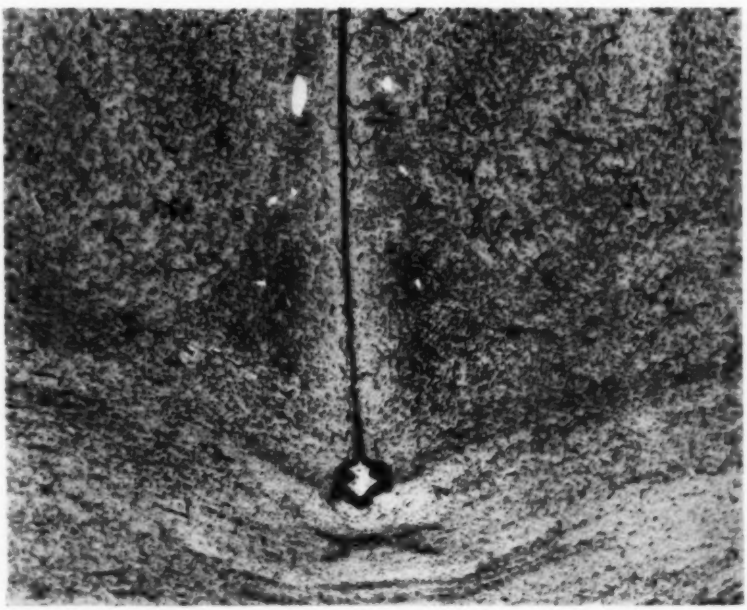

FIG. 4. Section through third ventricle and thalamus 30 hours after infection showing marked capillary and venous dilatation and engorgement (× 50).

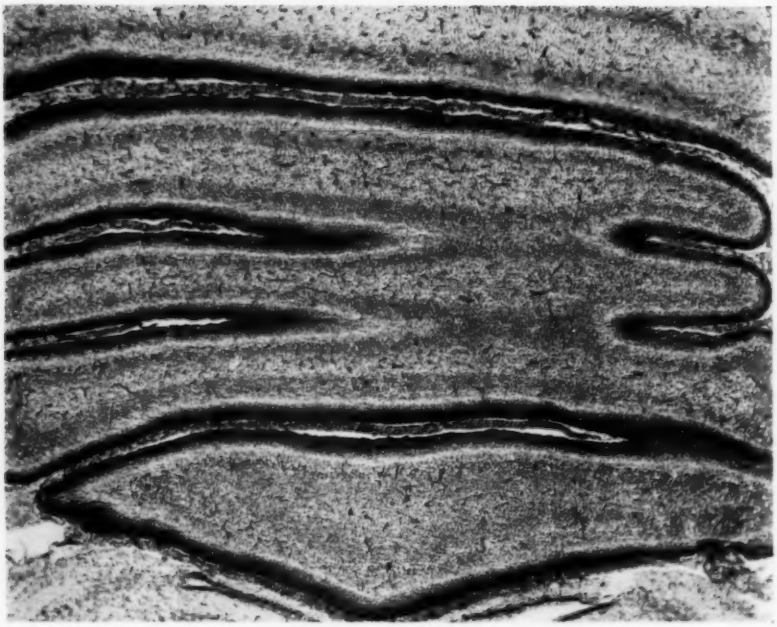

FIG. 5. Section through cerebellum 30 hours after infection showing generalized venous and capillary dilatation and engorgement (× 50).

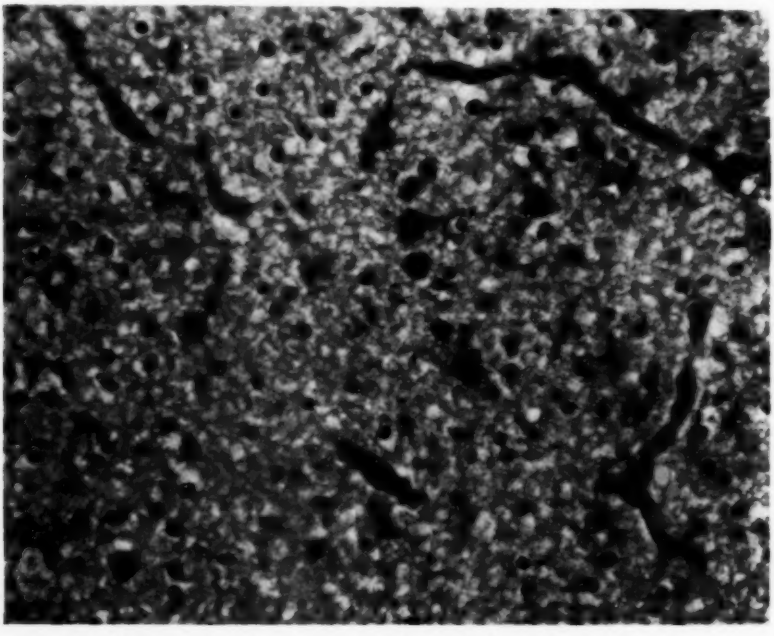

FIG. 6. Section through white matter of cerebrum 30 hours after infection showing capillary engorgement, pericapillary edema and glial swelling (× 500).

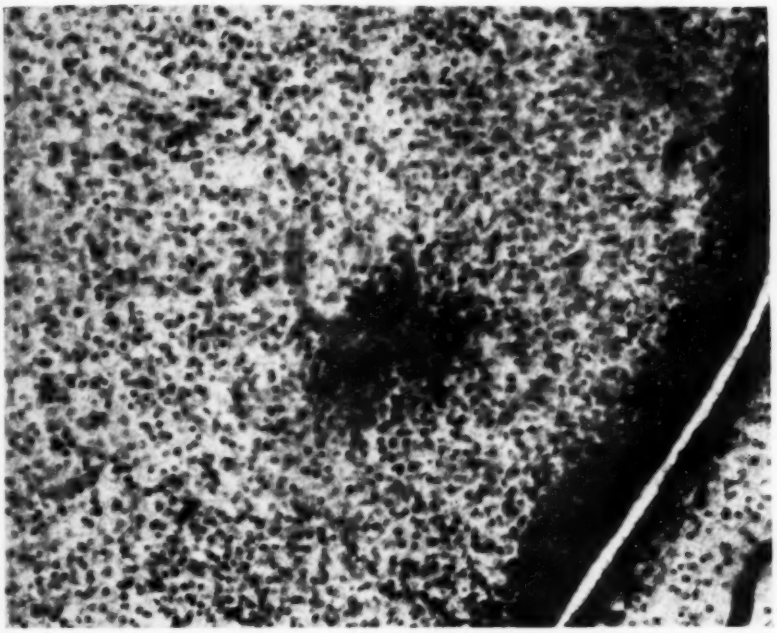

FIG. 7. Section through white matter of cerebrum 36 hours after infection showing capillary rupture (× 300).

lar hemispheres, next in cerebral and cerebellar cortex, and finally in thalamus and brain stem. In uninfected brains, or in brains removed shortly after infection, the capillary vascular network was inconspicuous. Twenty-four to 30 hours after infection, however, branching small blood vessels filled with erythrocytes were prominent (Figs. 4, 5 and 6). Capillary engorgement, endothelial swelling and blood stasis were followed by edema spreading out from perivascular regions into the ground substance (Fig. 6) and by capillary rupture (Fig. 7).

A single hemorrhage was observed in one chicken embryo 14 hours after infection (Fig. 8); this was an unusual occurrence. Multiple perivenous and pericapillary hemorrhages appeared 30 to 46 hours after infection (Fig. 9). These soon became coalescent and produced extensive distortion of brain architecture (Fig. 10). Plasma and blood also escaped into the ventricular system (Figs. 11 and 12) and subarachnoid and subdural hemorrhages appeared.

In the early stages of brain edema, swelling occurred around the nuclei of the oligodendroglia, and the ground substance became distended with edema fluid, producing a pavement-like appearance of the glia. Swelling of astrocyte

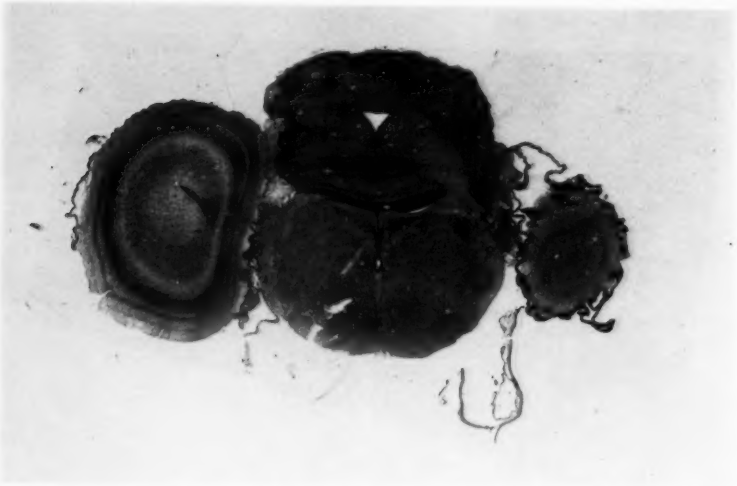

FIG. 8. Section through occipital poles, cerebellum and brain stem 14 hours after infection showing an early linear venous hemorrhage (× 12).

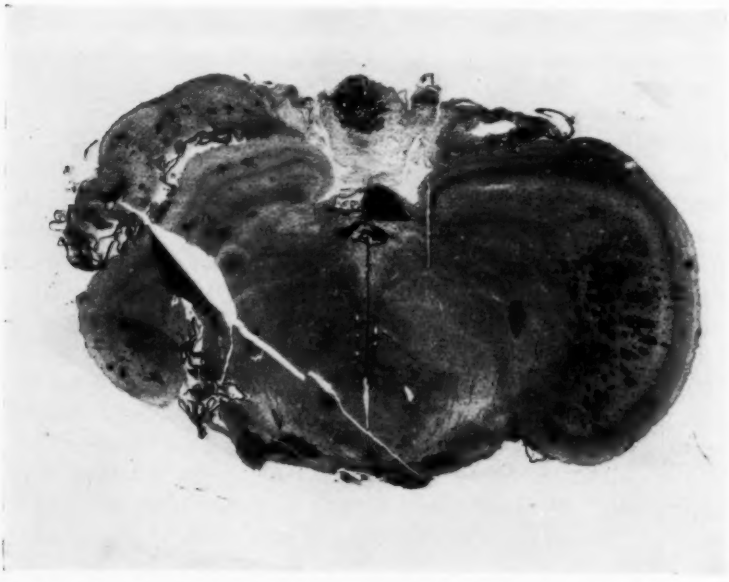

FIG. 9. Section through cerebrum, thalamus, and third ventricle 36 hours after infection showing multiple hemorrhages occurring predominantly in the white matter (× 12).

FIG. 10. Section through cerebrum, cerebellum and brain stem 46 hours after infection showing massive, coalescent hemorrhages with marked brain edema (× 12).

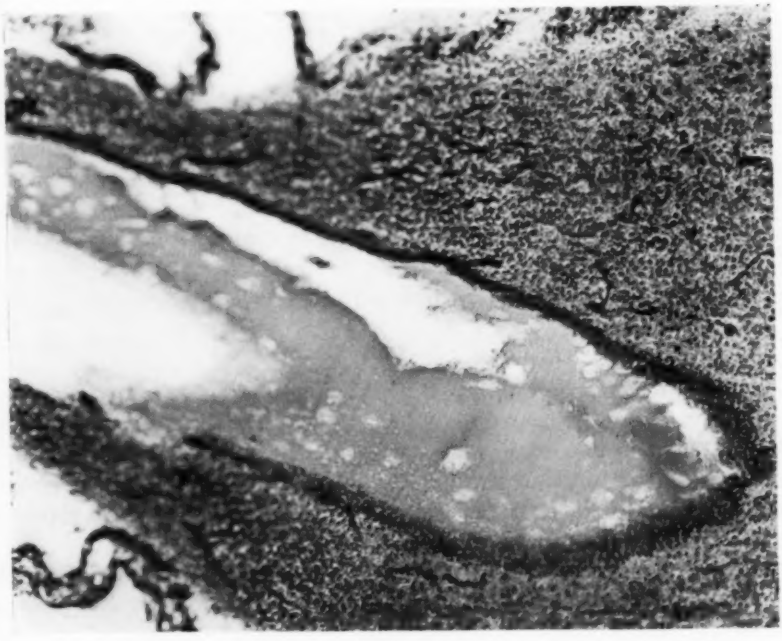

FIG. 11. Section showing escape of plasma into lateral ventricle (× 150).

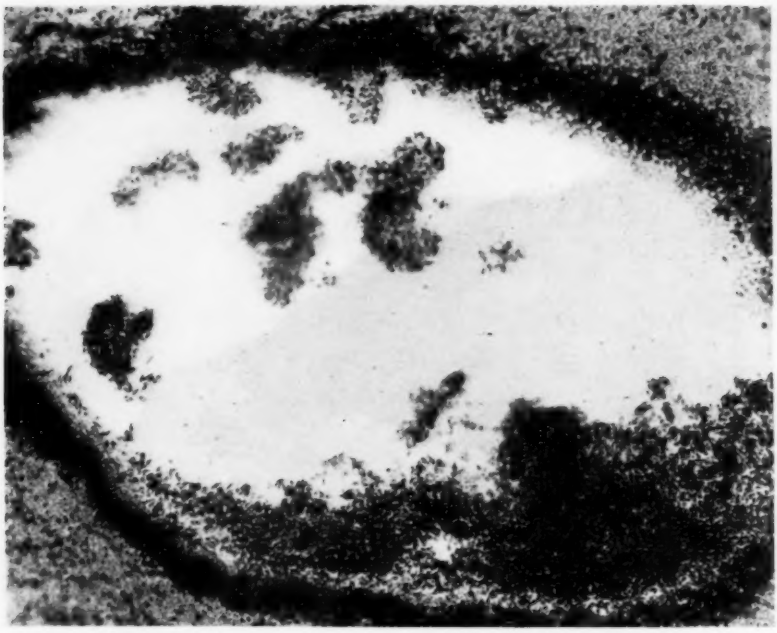

FIG. 12. Section showing intraventricular hemorrhage (× 150).

nuclei and astrocyte bodies, and occasional vacuolization of neurons were also observed.

At no time was there evidence of endothelial proliferation, inflammatory reaction in brain parenchyma or around blood vessels, or invasion by macrophages. In brains with massive hemorrhages, necrosis of neurons was seen throughout but this occurred only very late in the course of infection, apparently secondary to the vascular lesions. Only intact erythrocytes were seen in distended vessels and organized thrombi were not encountered.

DISCUSSION

The sequence of development of cerebral lesions in chicken embryos infected with "neurotropic" influenza virus suggests that the primary action of the virus in the brain is on blood vessels rather than on nerve cells. Only vascular lesions were noted until late in the course of infection when viral multiplication had passed its peak, and the necrosis of nervous tissue that occurred appeared to be secondary to extensive vascular lesions.

Although further studies are required to determine the exact mechanism by which influenza virus damages cerebral blood vessels, the histologic lesions developed in a sequence consistent with primary involvement of vascular

endothelium. The earliest changes noted were capillary distension and engorgement, and swelling of endothelial cells, followed by pericapillary edema, effusion of plasma, and finally rupture of the greatly distended capillaries. The appearance of well preserved erythrocytes within capillary lumens was more consistent with stasis than thrombosis. Involvement of capillaries and small veins throughout the brain and the absence of arterial occlusions and ischemic necrosis are taken as evidence that hemorrhages were not secondary to multiple emboli. It seems likely that damage to small blood vessels results from virus multiplication within endothelial cells, as postulated by Burnet and Fraser (5).

The reason hemorrhages occur predominantly in the brain is obscure. As noted in the preceding paper (4), growth curves of NWS virus in liver are identical to those in brain. Cerebral capillaries of the chicken embryo may be particularly vulnerable to distension and prone to rupture. The cerebral blood vessels are involved in many other viral infections of the central nervous system as evidenced by the frequent occurrence of small hemorrhages, perivasculitis and cerebral edema. Autopsies of patients with measles (6, 7), mumps (8) or vaccinial encephalomyelitis (9) have shown lesions predominantly of blood vessels supplying the white matter of the brain. Characteristic intranuclear inclusion bodies of fox encephalitis virus are found in endothelial cells of the brain, and not in nerve cells or endothelium of other tissues (10). Chicken embryos infected with pseudorabies virus show marked cerebral hemorrhages consistent with destruction of vascular endothelium (11). Distemper virus also localizes in cerebral vascular endothelium of ferrets (12) and dogs (13) as well as in nerve cells. Marked hemorrhages in brain and other tissues often occur in chicks and ducklings infected with Rous sarcoma virus (14), and in chicken embryos infected with certain of the encephalitis viruses (15, 16).

Myxoviruses other than influenza can cause cerebral hemorrhages in immature hosts. The histopathology of mumps encephalitis of suckling hamsters and mice suggests that the virus acts directly on blood vessels of brain (17). Certain strains of Newcastle disease virus produce hemorrhages in the brains of chicken embryos (18), similar to those of influenzal hemorrhagic encephalopathy. As previously noted (4, 5), chicken embryos infected with non-neurotropic strains of influenza virus also occasionally develop hemorrhagic brain lesions. Therefore, no direct correlation is evident between infectivity of a virus for nervous tissue and its capacity to affect cerebral blood vessels.

SUMMARY

A detailed histopathologic study was made of the brains of chicken embryos infected intravenously with a "neurotropic" variant (NWS) of influenza A virus. A diffuse, progressive hemorrhagic encephalopathy developed, charac-

terized by dilatation and congestion of capillaries, endothelial swelling, stasis of blood, perivascular edema, swelling of ground substance, and finally capillary rupture and extensive hemorrhage. Inflammation was conspicuously absent and necrosis of nervous tissue occurred only late in the course of infection secondary to vascular damage.

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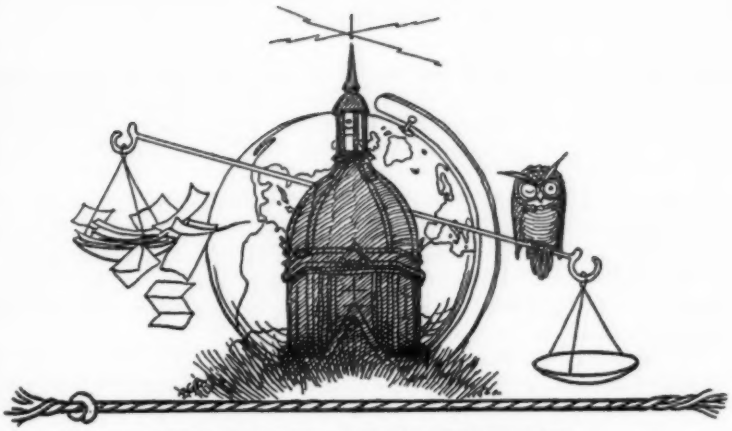

BOOK REVIEWS

Genetic Resistance to Disease in Domestic Animals. By FREDERICK BRUCE HUTT. 198 pp., $3.50. Cornell University Press, including Comstock Publishing Associates, Ithaca, N. Y.

The Professor of Animal Genetics in the New York State College of Agriculture of Cornell University has written a delightful and informative monograph which although concerned particularly with resistance to infectious disease is an excellent survey of all genetic disease in domestic animals. It is an admirable introduction to the field of comparative genetic pathology. In addition to the large portion of the monograph devoted to resistance to bacterial, protozoal and viral disease, a chapter discusses some of the clearly hereditary disorders, such as achondroplasia, epithelial defects (possibly comparable to epidermolysis bullosa congenita), the "short-spine" condition of cattle in which only the axial skeleton is affected, hairlessness of cattle, hereditary diseases of the central nervous system, and so on. A chapter entitled "Aberrant Metabolism" discusses biochemical genetics in domestic animals. Another called "Ways of Resisting" reviews some of the known mechanisms of genetic protection against disease.

The medical geneticist will be interested to find parallels to his own experiences with genetic diseases in man. Hutt cites several instances of what in human populations would probably be referred to as "balanced polymorphism". Just as the heterozygote for the sickle cell trait has an advantage in malarious areas over both types of homozygote, the "yellow" mouse heterozygote is more resistant to mammary carcinoma than its white sister, although the homozygous form of "yellow" is lethal (p. 27). The platinum fox certainly is considered by furrier and female fancier to have an advantage over the standard silver fox of which it is a heterozygous mutant but the homozygous state for "platinum" is lethal. Hutt cites several examples of hereditary syndromes which probably represent manifold expressions ("pseudo-pleiotropy") of a single gene which controls some biochemical process implicated in several areas: in some gray breeds of sheep, such as the Karacul, the animal that is homozygous for gray has also a peculiar disorder of the gastrointestinal tract (p. 26). The great majority of genetic disease in domestic animals behave as Mendelian recessives. Obviously dominant traits are easily eliminated whereas recessive genes may become widely disseminated. Muller estimates that on the average man carries, in heterozygous form, about eight different genes for grave hereditary "recessive" diseases. Some indication of this incidence can be obtained from the study of highly inbred human populations (i.e., those with a high "consanguinity rate"). By actual mating experiments six

randomly selected bulls of various dairy breeds were found to carry two lethal characters and genes for six other simple defects (p. 29). The desirability of recognizing genetic carriers of recessive diseases (i.e., heterozygotes) is appreciated by the animal geneticist; examples such as the disturbing dwarf condition of Aberdeen Angus and Hereford cattle are cited (p. 31).

Porphyria in cattle and swine, purine metabolism in the Dalmatian, and "yellow fat" in sheep and rabbits are discussed in the section on biochemical genetics. Hutt describes the interesting recessive mutation in sheep which results in photosensitization because the liver of the affected animal is unable to dispose of phylloerythrin, a digestion product of chlorophyll (p. 128). Fascinating genetic differences in the requirement and utilization of riboflavin and thiamin are described (p. 131–139).

Two sections of the book which are perhaps most valuable as illustrations of the interplay of environment and genome are those entitled "On Coping with the Environment" and "Ways of Resisting". Animals are maintained under more diverse environmental circumstances than obtain for man. The expression of a given gene may be quite different under different environmental conditions, as illustrated by the many examples provided. Twin studies in animals, for dissecting out the separate effects of nature and nurture, are discussed.

The gene-determined mechanisms of resistance to disease in animals are many. Although most are still unknown, those which have been elucidated point to the importance and fascination of this area of investigation. In guinea pigs a genetic defect in blood complement (inherited as a recessive) determines low resistance to infection (p. 147). The fact that White Leghorn chicks are more resistant to "pullorum disease" than Rhode Island Reds is related in some way to the fact that after hatching the White Leghorn chick is able to attain the normal adult temperature (105–109°F.) more quickly (p. 157). (Pasteur provided a classical demonstration that the fowl's immunity to anthrax results from its high body temperature. Dunking in cold water would break the immunity.) ***Klendusity*** (from Gr. *kle* "to bar", and *endusis*, "entry") is a term applied to the resistance to infection which results from anatomical or physiologic characteristics which prevent entry of the agent (p. 155). The classical example is the striking resistance of Zebu cattle to tick-borne disease, as compared with European cattle. It is not demonstrated that the Zebus have greater ability to resist disease once infected. Rather, the resistance is a matter of the ability to escape infection because of the physical character of the Zebu hide which admits an immunizing but not infecting dose of organisms.

Hutt's monograph is eminently readable. Such a relatively small book can not be exhaustive in its treatment of such a large subject. However, bibliographic references are numerous and seemingly well chosen. The book is spiced with "digs" at veterinary students, practicing veterinarians, government veterinarians, and animal breeders. Scattered through it and concentrated in the final chapter is a lament that more use is not made of genetic resistance to disease and the breeding of resistant stock, rather than relying only on vaccines, "slaughter of the innocents", etc.

It should be evident from the samplings provided, that this book will be of great usefulness to the medical geneticist.

VICTOR A. MCKUSICK

Clinical Enzymology. Edited by GUSTAV J. MARTIN. 241 pp., $6.00. Little, Brown & Co., Boston, Mass.

The intriguing title of this volume, "Clinical Enzymology," might more appropriately have been "Enzymology for Clinicians," but even with this modification, it would suggest

a greater scope of content than this work actually offers. The author, Gustav J. Martin, is Research Director of the National Drug Company of Philadelphia, which markets trypsin for parenteral use. Much of the material concerns the nature and use of enzymes such as trypsin, hyaluronidase, streptokinase and the ribonucleases. A title such as "The Clinical Use of Parenteral Enzymes" might have been appropriate except for two reasons: 1) There probably is not enough to say on the subject to warrant the writing of a whole book, and 2) in the work under discussion, there are a few digressions into other fields, such as enzyme inhibitors, and the "Diagnostic Use of Enzymes," i.e., the measurement of blood concentrations of enzymes for diagnostic purposes. Unfortunately none of the sections are complete enough to stand entirely alone, but they do contain sections worthy of review, particularly for purposes of reference.

The treatise starts out with a broad discussion of "Protein Biology," describing some general characteristics of proteins and enzymes and general concepts concerning their nature. The reviewer found this introduction generally interesting. It is evidently not intended to take the place of a more comprehensive treatment of protein chemistry. Some parts of this section however, seem rather speculative. The discussions become quite wordy and sometimes vague (at least to me). For example, on page 29 we find the sentence, "Life is a vortex in the earthstream of physiologically designed atomic and molecular units." Succeeding sections discuss the chemistry (by M. J. Sullivan) and biochemistry (by J. M. Beiler) of enzymes used clinically, and the parenteral use of these enzymes (by H. Tanyol *et al.*). The final sections are the Diagnostic Use of Enzymes (by G. V. Rossi), Polymerases in Biology, and a Recapitulation (both by G. J. Martin).

In summary, the reviewer feels that although this book could serve as a useful reference text for certain specific purposes, it is too much of a hodge-podge and too incomplete to be of the greatest use for either the chemist or the clinician. It might be said, however, that the writing of a volume in this field must be a difficult task, and the author should be given due credit for the effort he has made. A great deal of literature has been reviewed.

WILLIAM P. MCCANN

Ulcerative Colitis. By HARRY E. BACON. 395 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

At the time of writing this book the author had had experience with 440 cases of ulcerative colitis. One hundred and eight of these patients were operated upon. The work is a thorough, careful review of the nature of the disease and its treatment. His basic thesis is that once ulcerative colitis is established and severe, the patient will ultimately come to colectomy and that this colectomy should be a total colectomy with abdomino-perineal resection, and, within reason, the sooner the better. With this opinion the present reviewer is in entire agreement, although accustomed to making a more formal apology for having no better treatment for a diseased organ than its removal. While agreeing, too, with the author's exasperation with those physicians who consider any postponement or avoidance of operation a triumph, even if it is at the cost to the patient of lifelong debility, I would prefer to see the thought processes of such physicians less sarcastically attacked.

From the standpoint of etiology, nothing new is offered. The development of cancer in the colon of patients with ulcerative colitis is well documented from the author's own experience and the reported literature. It will be difficult for any reader, after reviewing the masses of statistics offered, and reading the case citations, to escape the conclusion that patients with ulcerative colitis are considerably more liable to the development of cancer of the colon than other patients, and that the duration of the disease is the single most important factor, and finally, that this is particularly so in the younger patients. As to

whether he feels that these carcinomas develop from pseudo polyps or independently of them, I have had great difficulty in determining from Bacon's analysis. He seems content to leave the status of the matter at the point at which it is admitted that the pseudo polyps are not true adenomata and therefore not necessarily connected with the development of cancer, but that the increase in numbers of pseudo polyps is associated with increasing likelihood of developing carcinoma.

With the author's general techniques and method of handling these patients I find myself very largely in agreement, except for occasional details. If the book has any serious fault it is that the descriptions of operative procedures, which differ relatively little from one to another, are repetitious and given in too great and rather boring detail. The same may be said of the details of pre-operative and post-operative management. On the other hand, these sections, particularly that on the care of ileostomies should be required reading for the nurses and house staff in surgical clinics treating such patients. Useful also are the lists of types of ileostomy equipment and the addresses of the manufacturers supplying them.

Bacon makes a strong case for the essential insensitivity of the disease to all forms of treatment available up to the present, although he describes, in detail, the various drugs and regimens which have been advised from time to time. He apparently has mixed feelings towards the use of the steroid hormones and seems enthusiastic about them only in connection with fulminating cases in which the disease has at times been strikingly affected in a very short space of time.

Bacon ably supports the thesis that once an ileostomy is required, total colectomy will almost inevitably be required and that the mortality of ileostomy is much greater than the mortality of ileostomy plus colectomy, and that there is little or no place today for the operation of ileostomy alone in the treatment of ulcerative colitis. He is perhaps not as forceful in advocating the definitive operation in fulminating cases as in the well established chronic cases.

MARK M. RAVITCH

Clinical Hematology, 4th edition. By MAXWELL M. WINTROBE. 1185 pp., $15.00. Lea & Febiger, Philadelphia, Pa.

This text remains probably the single most complete treatise in its field. The author surveys not only known basic facts concerning the physiology, embryology and biochemistry of the hematopoietic system but also details, methods of laboratory determination, and the clinical disorders involving primarily the blood or blood forming organs. The present edition is both a revision and expansion of the 3rd edition published in 1951 and incorporates many of the new advances made in these fields in recent years. As is inevitably true of such a text, some sections have already been made incomplete due to more recent discoveries. For instance, although the author describes clearly and succinctly the experimental work on animals indicating the importance of pyridoxine in erythrogenesis, this volume was prepared before the recognition of a clinical state of hypochromic microcytic anemia apparently due to spontaneous pyridoxine deficiency in humans.

These minor deficiencies regarding the most current advances in no way, however, detract from the usefulness and completeness of this volume as a learned and readable survey of the entire field of hematology. As was true of his earlier volumes, the bibliography for each section has been carefully selected and will provide excellent references for those interested in pursuing more thoroughly any one of many subjects.

There is only one minor criticism which one could fairly advance of the contents of this volume. The author has again included his discussion of myelofibrosis (agnogenic myeloid

metaplasia) in the section on myelophthisic anemias. The variety of names by which this disorder is known has long been a source of confusion to all and the author attempts to point out that he feels these cases do represent a single disease state. However, the running discussion of myelofibrosis inserted into the description of myelophthisic anemias due to such causes as metastatic carcinoma, has proved a source of confusion to both students and practitioners and may, in this writer's opinion, prevent them from recognizing the former disorder as a distinct clinical entity.

PATRICIA A. MCINTYRE

The Healing of Wounds. Edited by MARTIN B. WILLIAMSON. 202 pp., $7.00. The Blakiston Division, McGraw-Hill Book Co., New York, N. Y.

This small volume contains a wealth of material and represents the work of a number of contributors to a symposium on this subject. It is edited by Dr. Williamson who is presently Professor of Biochemistry at the Stritch School of Medicine of Loyola University in Chicago. His particular interest has been the metabolic changes involved in the healing of wounds and he has been a significant contributor to the knowledge on the subject for the past ten years or more. There are other sections on the role of the ground substance, methionine, sulfur, vitamin and hormonal influences and collagen. As the editor points out, "basic information about the processes involved in the healing of wounds has accumulated to a remarkable degree in recent years". It is interesting to the reviewer that thus far none of this information has significantly altered either the production or treatment or suturing of wounds. Surgeons through careful observation, beginning notably with Lister and Halsted, have known for a long time how to deal with wounds without completely understanding all the basic concepts of healing. To say this, however, is not to imply that there will not be a change in the future in this regard.

EDWARD S. STAFFORD

BOOKS RECEIVED FOR REVIEW

Action of Radiation on Tissues. An Introduction to Radiotherapy. By A. LACASSAGNE AND G. GRICOUROFF. Translated by Clarence C. Lushbaugh and Gretchen R. Riese. 199 pp., $6.25. Grune & Stratton, Inc., New York, N. Y.

An Atlas of Airborne Pollen Grains. By H. A. HYDE AND K. F. ADAMS. 112 pp., $10.00. St. Martin's Press, New York, N. Y.

Atrial Arrhythmias, Digitalis and Potassium. By BERNARD LOWN AND HAROLD D. LEVINE. 222 pp., $6.90. Landsberger Medical Books, New York, N. Y.

Causation of Cancer. British Medical Bulletin, Vol. 14, No. 2, May 1958. 196 pp., $4.00. Medical Department, The British Council, London, England.

The Cerebrospinal Fluid. Production, Circulation and Absorption. Ciba Foundation Symposium. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 335 pp., $9.00. Little, Brown & Co., Boston, Mass.

Communication for Nurses. By FLORENCE K. LOCKERBY. With Foreword by Lucille S. Spalding. 175 pp., $3.75. The C. V. Mosby Co., St. Louis, Mo.

Diagnostic Laboratory Hematology, 2nd edition. By GEORGE E. CARTWRIGHT. 250 pp., $6.75. Grune & Stratton, New York, N. Y.

Electrocardiography. By MICHAEL BERNREITER. 134 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Gestation. Transactions of the Fourth Conference March 5, 6 and 7, 1957. Edited by CLAUDE A. VILLEE. 216 pp., $4.50. The Josiah Macy, Jr. Foundation, New York, N. Y.

Illustrated Preoperative and Postoperative Care. By PHILIP THOREK. 98 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Medical Electrical Equipment. Advisory Editor, ROBERT E. MOLLOY. 312 pp., $15.00. Philosophical Library, New York, N. Y.

Modern Clinical Psychiatry, 5th edition. By ARTHUR P. NOYES AND LAWRENCE C. KOLB. 694 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Operating Room Manual. A Guide for O.R. Personnel. By MARY ELLEN YEAGER. 213 pp., $4.50. G. P. Putnam's Sons, New York, N. Y.

Orthopedic Diseases. By ERNEST AEGERTER AND JOHN A. KIRKPATRICK, JR. 602 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

The Psychology of Medical Practice. By MARC H. HOLLENDER. 276 pp., $6.50. W. B. Saunders Co., Philadelphia, Pa.

THE EFFECT OF THYROID HORMONES AND TEMPERATURE ON THE KINETICS OF CONTRACTION AND RELAXATION OF VENTRICULAR HEART MUSCLE[1, 2]

WILLIAM R. BREWSTER, JR., JAMES P. ISAACS,[3] PATRICIA R. OSGOOD, ANNE M. NYLANDER AND RAYMOND Y. W. CHOCK

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A fundamental problem in muscle physiology has been the determination of the relationship of muscle metabolism to function. As summarized by Ramsey (1, 2), two opposing views are currently held concerning the dependence of contraction and relaxation upon the transfer and utilization of chemical bond energy. The first implies that chemical bond energy is expended during the production and perhaps maintenance of the contracted state of muscle (3, 4, 5, 6). This view has been associated with the concept that relaxation of striated muscle is essentially a passive process not dependent upon metabolic processes and accomplished by internal elastic or external extending forces. The second major concept relating energy transfer to configuration has viewed relaxation as an active process in which bond energy or electron transfer is essential. The relaxed state from this viewpoint has been characterized as that of a high potential internal energy affected by the transfer of the energy of metabolism (1, 2, 7 to 12). Associated with this viewpoint is the concept that contraction is a physical process not requiring electron or quantum energy transfer within the contractile system.

The kinetics of contraction and relaxation of ventricular heart muscle have been studied in the intact animal in which metabolic rate has been maximally varied both by alterations in thyroid hormone concentrations and in temperature. The studies reported below indicate that major variations in the rate of metabolism are least manifested by changes in the kinetics of initial contraction of muscle. An inverse relationship exists between the metabolic rate and the duration of the contracted state or of relaxation.

[1] This work was completed by Dr. W. R. Brewster during the tenure of a Research Fellowship and an Established Investigatorship in the American Heart Association.

[2] This work was supported by grants-in-aid from the Massachusetts Heart Association, Wyeth Laboratories, Philadelphia, and under USAF Contract No. SRKB AF-49 (638)98, and AF 18 (600)-1258.

[3] John and Mary R. Markle Foundation Scholar in Medical Science. Department of Surgery, The Johns Hopkins University School of Medicine and Hospital.

METHODS

1. *Animals*

Seventeen mongrel male dogs varying in weight from 10 to 24 kilograms were used. Included were four athyroid dogs, eight euthyroid dogs, and five dogs which had been fed thyroid powder as described below.

2. *Preparation of Animals*

a. *Athyroid Dogs.* The athyroid state was produced by the combined use of total thyroidectomy and of I^{131}. The dogs received I^{131} in a dosage of 0.5 millicuries/kilogram of body weight at intervals of 6 and 12 weeks following thyroidectomy for the purpose of destruction of remaining thyroid and accessory thyroid tissue in the neck and upper mediastinum. All dogs were fed a low iodine diet composed of carbohydrate and of vegetable fats and proteins in order to eliminate the ingestion of thyroid hormones present in fish and animal proteins. The animals were studied 22 to 24 weeks after the initial I^{131} injection. All dogs were myxedematous in appearance. The mean serum protein bound iodine concentration of the group was 0.5 micrograms per cent.

b. *Thyroid Feeding.* Animals were fed thyroid powder daily calculated on the basis of 0.8 gm./kg. of body weight. Studies were done after nine to sixteen days of thyroid feeding. As previously reported, this regimen of thyroid feeding is sufficient to raise the serum protein bound iodine concentrations to average values of 16 to 18 micrograms per cent (13).

3. *Anesthesia*

All dogs were anesthetized with thiopental sodium for surgical preparation for the physiologic observations described below. Following surgical preparation and prior to control studies, the dogs were allowed to recover from the thiopental anesthesia to the extent that superficial reflexes were present and the animal would react to cutaneous stimuli with a motor response.

To prevent shivering during the period of cooling and rewarming, 0.125 mg./kg. of decamethonium bromide (C-10) was injected intravenously at 20 to 45 minute intervals. This dose of decamethonium had no observable effect upon the contractility of ventricular heart muscle.

4. *Determination and Control of Body and Heart Temperature*

Rectal, esophageal, and ventricular heart muscle temperatures were simultaneously determined utilizing thermistor resistance elements sutured in right and left ventricular heart muscle and inserted into both the esophagus and rectum of the animal.

Cooling and warming of the dogs over the temperature range of 42°C. to 17°C. was accomplished using an extracorporeal veno-venous shunt. Blood was removed from and returned to the inferior vena cava by an Olson pump. The rate of cooling and rewarming could be controlled both by a variation in the strike volume of the pump and by the temperature of the bath in which the external coils were immersed. For the purposes of this study, animals were slowly cooled and rewarmed at a rate not exceeding 1°C./15 min. At this rate of cooling or rewarming, temperature gradients between the heart and rectum or esophagus did not exceed 0.5°C.

5. *Direct Recording of Contraction and Relaxation of Ventricular Heart Muscle*

Recordings of isometric contraction and relaxation of right and left ventricular heart muscle were obtained utilizing strain gauge arches sutured to segments of both right and

left ventricular heart muscle as described by Boniface, Brodie, Walton (14) and Cotten (15, 16). The strain gauges were calibrated in terms of grams of weight exerting their force in parallel with the resistance element. Following suture of the arches, the length and tension of the muscle segment between the legs of the resistance arch was increased until a maximum contractile force was observed. Observations reported below on the contractile force and duration of events in the cardiac cycle are thus derived from isometric contraction and relaxation of the muscle segment sutured in parallel with the resistance element of the strain gauge arch. In addition to the force contribution from isometric contraction and relaxation, there is an isotonic contribution from the syncytium of muscle external to the strain gauge arch. This contribution cannot be quantitatively evaluated but is believed not to alter fundamentally the evaluation of the duration of phases of activity of ventricular heart muscle reported below.

6. *Intracardiac and Extracardiac Pressures*

Pulmonary arterial, left atrial, and left ventricular pressures were obtained via No. 10 polyethylene catheters placed indirectly through small vessels approached by thoracotomy. Right atrial and right ventricular pressures were obtained by catheters inserted through the external jugular vein. Femoral arterial pressures were obtained through a No. 10 polyethylene catheter ligated into the femoral artery. Full range and condenser integrated mean pressures were obtained with Sanborn electromanometers. The pressures were recorded with a four channel Sanborn oscillograph together with the strain gauge signals and an electrocardiogram obtained with one of the standard extremity leads.

Following thoracotomy for insertion of catheters, the chest was closed and an intrathoracic cannula was left in place for the purpose of establishing and maintaining a negative intrathoracic pressure by suction and water seal.

7. *Oxygen Consumption and Ventilation of Animals*

Animals were ventilated through a No. 35 Magill tube firmly tied in the trachea following tracheotomy. Mechanical ventilation was maintained throughout the course of all experiments. A system, described elsewhere (17), was used for the simultaneous intermittent positive pressure ventilation of the animals with an Emerson resuscitator and spirometric determination of the oxygen consumption.

8. *Blood Volume*

Blood drawn for samples and initially displaced into the extracorporeal pump was replaced by an equal volume of dextran.

9. *Calculations*

a. The body surface area in square meters was obtained with Meehs formula, using Rubner's constant for the dog, as follows:

$$\text{Surface area}/M^2 = 1.12\ (\text{weight/kg.})^{.67}$$

b. The oxygen consumption is corrected to standard temperature and pressure and is expressed in milliliters per minute per square meters of body surface area.

c. The temperature coefficients (Q10) values were calculated to express the ratio of the value of a particular parameter studied to its value at a temperature 10°C. lower and is defined by the equation:

$$Q10 = \frac{K_{(t-10)}}{K_{(t)}}$$

where t = temperature in degrees Centigrade and K = velocity constant of the reaction. Over the temperature range studied, the Q10 was derived from the equation:

$$\text{Log } Q_{10} \simeq \frac{10}{t_2 - t_1} \times \text{Log} \frac{K_2}{K_1}$$

d. The activation energy or u values were calculated from the Arrhenius equation relating reaction velocity to temperature as follows:

$$\frac{d \ln k}{dT} = \frac{u}{RT^2}$$

where u = activation energy (cal./mole)
k = velocity constant of reaction
T = absolute temperature
R = gas constant = 1.986 cal. deg.$^{-1}$ mole^{-1}

The above equation is also expressed as follows:

$$\ln \frac{K_1}{K_0} = \frac{\mu}{1.986}\left(\frac{(T_1 - T_0)}{T_1 T_0}\right)$$

Where K_1 and K_0 are reaction velocity constants at absolute temperatures T_1 and T_0 respectively, 1.986 = gas constant(cal. degree^{-1} mole^{-1}) and μ the activation energy.

10. *Conversion Ratios to Correct Observed Values of the Contractile Force and Rates of Contraction and Relaxation to Values of Equivalent Magnitude*

Prior to suture to ventricular heart muscle, the strain gauges were calibrated in terms of the grams of force required for a standard oscillographic deflection and the duplicates of records below indicate the grams of force producing a given deflection. In suturing the strain gauges, there was a lack of uniformity in the lengths of muscle segments, a variation in the depth to which sutures were placed, and unequal orientation of the gauges relative to the longitudinal axes of the fibers of the segment. This resulted in a variation in the absolute contractile force among the different animals. It was thus not possible to compare the contractile force directly. Since the purpose of the work was to compare the effects of changes in temperature upon the initial and total contractile force in the same animal and to obtain average changes in each group of animals, the values for the contractile force and for the rates of contraction and relaxation were *normalized* about a value of the rate of initial contraction. This was done by correcting all rates of initial contraction in all dogs to 2000 grams/sec. at 37°C. All tensions and rates of contraction and relaxation were then multiplied by the factor (example below):

$$\frac{\text{2000 gms./sec. at 37°C.}}{\text{Observed Rate of Contraction at 37°C.}}$$

Observed Values at 37°C.

	Initial Contractile Force (gms.)	Total Contractile Force (gms.)	Rate Contraction (gms./sec.)	Rate Relaxation (gms./sec.)
Dog A	120	144.5	2780	2020
Dog B	107.5	113.0	1990	1720
Dog C	82.0	82.0	1235	1360
	Normalized Values at 37°C.			
Dog A	86.4	104.0	2000	1454
Dog B	108.6	114.1	2000	1737
Dog C	120.5	120.5	2000	2203

Corrections: Dog A $= \frac{2000}{2780} = 0.72$ *Dog B* $= \frac{2000}{1990} = 1.01$ *Dog C* $= \frac{2000}{1235} = 1.62$

Since it is the ratio of the changes in contractile force that is important in calculations of the Q10 and activation energy, these values were not influenced by the above conversion of data relating to contractile force. The normalization process merely permitted the assessment of changes in force and rates by the use of average values.

RESULTS

Terminology

Isometric contraction and relaxation displayed four distinct phases for analysis as shown in Figure 1: (1) Initial contraction, characterized by a rapid and near maximal increase in tension. The time of initial contraction is designated by AX, the initial contractile force by BX, and the maximum rate of initial contraction by the tangent to the maximum slope AB. (2) The contracted state, during which a further slow increase in tension may occur. The duration of the contracted state is designated by XY. The total contractile force is the tension at the termination of this phase, designated by CY. The "supercontraction rate," designating the maximum rate of increase in tension during this phase is the tangent to the slope BC. (3) Relaxation. The time required for isometric relaxation is indicated by YD and the maximum rate of relaxation by a tangent drawn in parallel to the steepest portion of the slope CD. The end of relaxation was determined by the point of inflection of the descending curve at or near the baseline. (4) Relaxed state, designated by DA. In all records time is constant at 1 mm. = 0.01 secs.

Time Relationships in Strain Gauge and Ventricular Pressure Recordings

In six dogs ventricular pressures were recorded simultaneously with the ventricular isometric myograms. Figure 2 demonstrates that the time relationships of initial contraction and the duration of the contracted state are identical with the phases of isometric ventricular contraction and ventricular systolic ejection respectively. Although initial diastolic pressure levels are reached prior to complete isometric relaxation, relaxation rate changes are seen to be accompanied by parallel changes in the rate of decrease in the ventricular pressure. The close correspondence of the time relationships for total ventricular activity as recorded by the ventricular pressures and that of isometric contraction and relaxation of segments of ventricular muscle in the intact animal justifies the analysis of the latter for an understanding of kinetic events in ventricular muscle as a whole.

TYPICAL CURVE OF CONTRACTION AND RELAXATION

AX= TIME OF INITIAL CONTRACTION (seconds)
XY= DURATION OF CONTRACTED STATE (seconds)
YD= RELAXATION TIME IN SECONDS
XB= INITIAL CONTRACTILE FORCE (grams)
YC= TOTAL CONTRACTILE FORCE (grams)
TANGENT OF MAXIMUM SLOPE AB = RATE OF CONTRACTION (gms/sec.)
TANGENT OF MAXIMUM SLOPE CD = RATE OF RELAXATION (gms/sec.)
TANGENT OF MAXIMUM SLOPE BC = "SUPERCONTRACTION" RATE

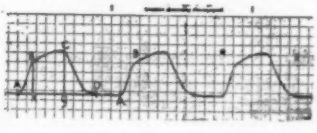

FIG. 1. Typical curve of contraction and relaxation.

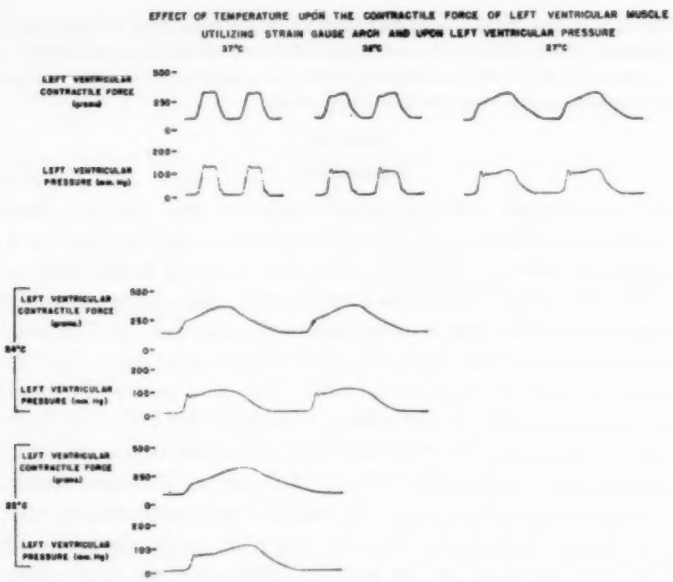

FIG. 2. Time relationships in simultaneous strain gauge and ventricular pressure recordings. Paper speed: 1 mm. = 0.01 seconds.

Effect of Temperature and Thyroid Hormone on Metabolic Rate

The rate of metabolism of the intact animal and of intact and isolated organs and tissues has been demonstrated to show a dependence upon temperature that is characteristic of chemical reactions in which there is electron transfer and a change in oxidation state. In accordance with van't Hoff's rule, the rate of such reactions generally increases two to threefold with a rise in temperature of 10°C., this change in the rate being customarily expressed by a Q10 of 2.0 to 3.0 (18, 19, 20, 21). The temperature coefficient or Q10 expresses a general correlation with the activation energy values in relating a change in the number of active molecules with an energy content great enough to react upon collision or to undergo a change in molecular configuration (22, 23, 24, 25). It should be noted that the activation energy values have been calculated from the data obtained for their general interest and are not meant to imply, in the pure sense, a particular reaction in a complex catenate of enzymatically catalyzed reactions.

In the intact animal, variations in thyroid hormone concentration are recognized to cause, in general, parallel changes in both body and heart metabolic rates (26, 27). The magnitude of effect of thyroid hormones and temperature upon the rate of tissue metabolism should be manifested in parallel changes in

the kinetics of those phases of activity of ventricular heart muscle which are directly linked to chemical reactions in which bond energy or electron transfer is directly regulated by either thyroid hormone or temperature. In the figures below, Arrhenius plots of data show the physiological parameter during various phases of heart muscle kinetics, plotted on a logarithmic scale against the reciprocal of the absolute temperature.

Initial and Total Contractile Force and Time

Within each group of animals the total contractile force remained unchanged over the range of temperatures studied. The *force* of initial contraction (Figs. 3a, 3b, and 3c) showed in general a small decrease with a lowering of temperature in all groups. The Q10's varied from 0.9 to 1.25 over the temperature range of 39° to 29°C. and from 1.10 to 1.48 between 39° to 24°C.

Figures 4a, 4b, and 4c demonstrate that there was a small increase in the *time* required for initial contraction with a lowering of temperature, with Q10 values varying from 1.09 to 1.38. (u = 2,600 to 6.040 calories/mole).

In view of the major changes that are observed below in the duration of the contracted state, it is important to note that a near maximum increase in tension had occurred during initial contraction at all temperatures and that the prolongation of the contracted state of muscle did not represent a slowing of the process of initial contraction.

Duration of the Contracted State

In all groups of dogs, there was an exponential increase in the duration of the contracted state when related to the reciprocal of the absolute temperature (Fig. 5). The duration of the contracted state was significantly longer in the athyroid animals at any given temperature than in the euthyroid and thyroid fed animals. The differences in the duration of the contracted state of the euthyroid and thyroid fed animals were not significant in this series, although there was a slightly shorter duration of the contracted state in the thyroid fed group. The Q10 values for this phase varied from 2.7 to 3.11 (u = 18,390 to 21,020 cal./mole). The significance of these values is the indication that an active transfer of chemical bond energy is essential to terminate contraction and initiate relaxation.

Time Required for Relaxation

The time required for relaxation was found (Figs. 4a, 4b, 4c, and Fig. 6) to vary with thyroid hormone concentration and temperature in the same qualitative manner as the duration of the contracted state. A similar exponential increase was found with a lowering of temperature. The Q10 values for relaxation varied from 3.09 to 3.40 (u = 20,860 to 22,100 cal./mole). The

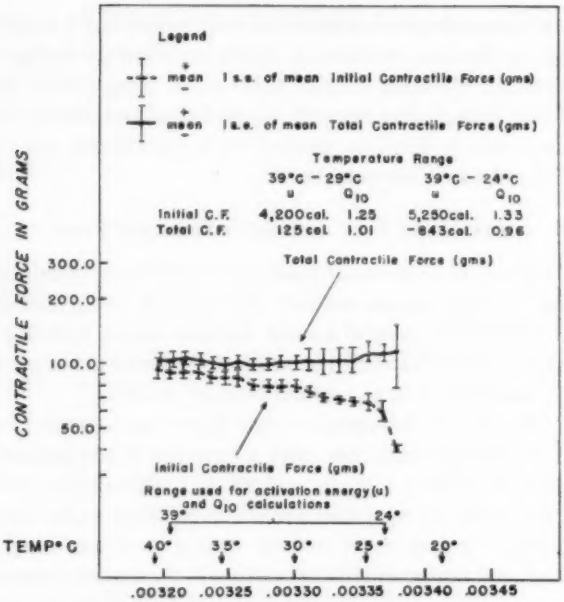
Legend
mean ± s.e. of mean Initial Contractile Force (gms)
mean ± s.e. of mean Total Contractile Force (gms)
Temperature Range
39°C - 29°C
u
Q10
39°C - 24°C
u
Q10
Initial C.F. 4,200cal. 1.25 5,250cal. 1.33
Total C.F. 125cal. 1.01 -843cal. 0.96
CONTRACTILE FORCE IN GRAMS
300.0
200.0
100.0
50.0
Total Contractile Force (gms)
Initial Contractile Force (gms)
Range used for activation energy (u) and Q10 calculations
39°
24°
TEMP°C
40°
35°
30°
25°
20°
.00320 .00325 .00330 .00335 .00340 .00345
1/TEMP. ABS.

FIG. 3a

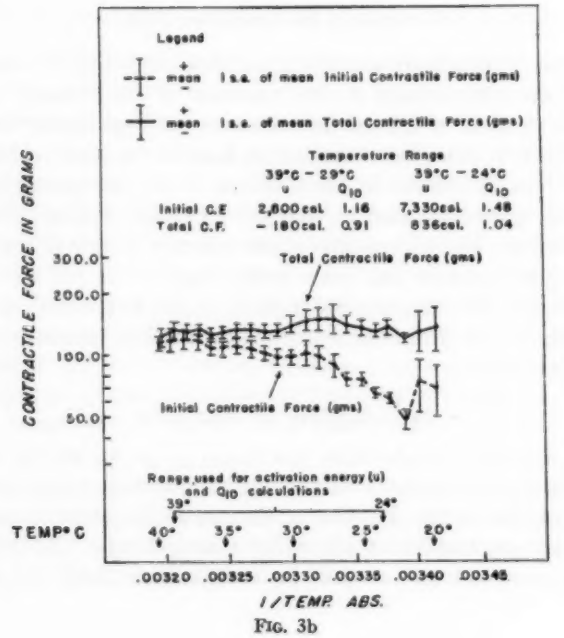
Legend
mean ± s.e. of mean Initial Contractile Force (gms)
mean ± s.e. of mean Total Contractile Force (gms)
Temperature Range
39°C - 29°C
u
Q10
39°C - 24°C
u
Q10
Initial C.F. 2,800cal. 1.16 7,330cal. 1.48
Total C.F. -180cal. 0.91 836cal. 1.04
CONTRACTILE FORCE IN GRAMS
300.0
200.0
100.0
50.0
Total Contractile Force (gms)
Initial Contractile Force (gms)
Range used for activation energy (u) and Q10 calculations
39°
24°
TEMP°C
40°
35°
30°
25°
20°
.00320 .00325 .00330 .00335 .00340 .00345
1/TEMP. ABS.

FIG. 3b

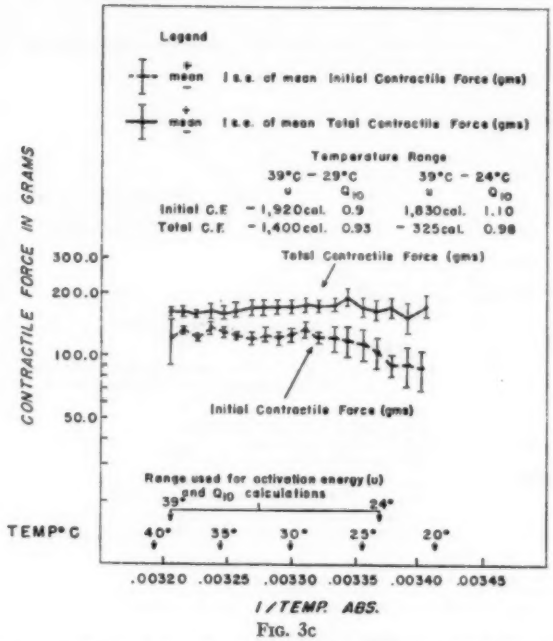

FIG. 3c

FIGS. 3a, 3b, 3c. Arrhenius plot of the relationship between initial and total contractile force in thyroid fed, euthyroid, and athyroid dogs over the temperature range of 39° to 24°C. All figures include the mean and ± one standard error of the mean.

athyroid animals demonstrated the slowest rate of relaxation at any given temperature when contrasted to the euthyroid and thyroid fed animals.

Oxygen Consumption and Heart Rate

These Q10 values appear to indicate that the initiation and completion of relaxation is related to an active metabolic process. As such, a distinct relationship should exist between the kinetics of relaxation and the rate of oxidative metabolism. In agreement with data presented by other workers (18, 22, 23, 25) the oxygen consumption of all groups of animals demonstrated an exponential relationship when related to the reciprocal of the absolute temperature (Fig. 7). Q10 values for total metabolic rate varied from 2.07 to 2.39 and are typical of the effect of temperature upon the rate of oxidative metabolism found, not only for the whole body, but for isolated and intact organs and tissues (17, 19, 20, 21). It should be noted that, at all temperatures, there was a significant difference between the oxygen consumption of the athyroid, euthyroid, and thyroid fed animals. Furthermore, if one compares Figures 5, 6, and 7, it will be noted that there is an inverse relationship between the oxygen consumption and either the duration of the contracted state or time required for relaxation.

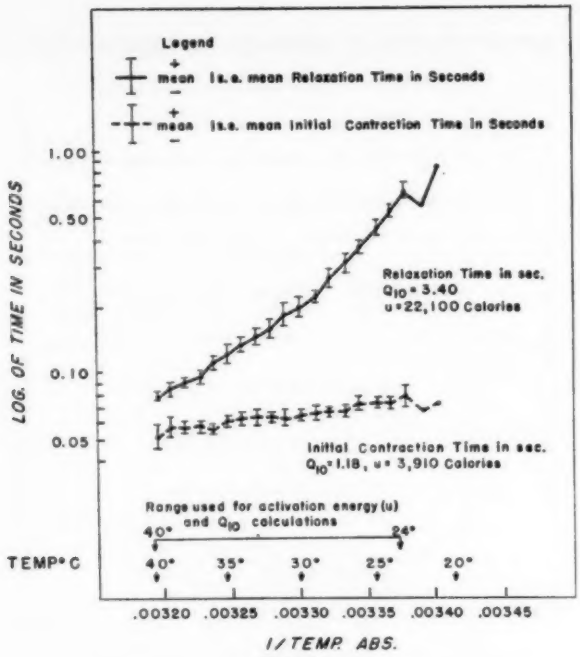
Legend
mean ± s.e. mean Relaxation Time in Seconds
mean ± s.e. mean Initial Contraction Time in Seconds
LOG. OF TIME IN SECONDS
1.00
0.50
0.10
0.05
Relaxation Time in sec.
Q10 = 3.40
u = 22,100 Calories
Initial Contraction Time in sec.
Q10 = 1.18, u = 3,910 Calories
Range used for activation energy (u) and Q10 calculations
40°
24°
TEMP° C
40°
35°
30°
25°
20°
.00320 .00325 .00330 .00335 .00340 .00345
1/TEMP. ABS.

FIG. 4a

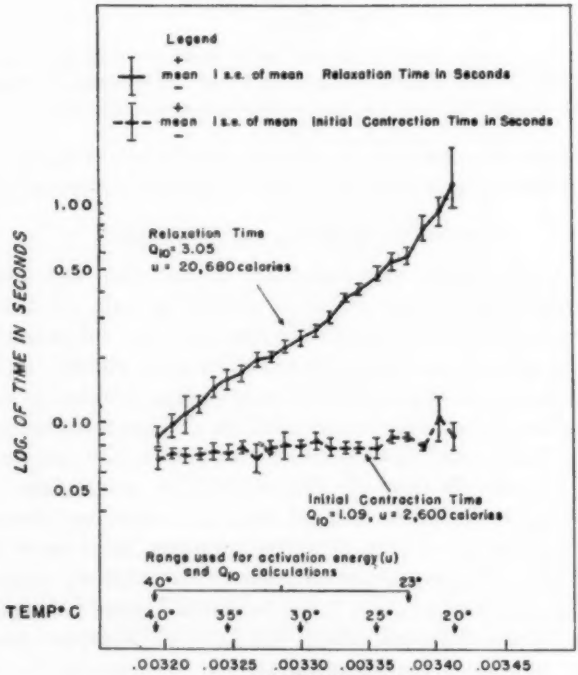
Legend
mean ± s.e. of mean Relaxation Time in Seconds
mean ± s.e. of mean Initial Contraction Time in Seconds
LOG. OF TIME IN SECONDS
1.00
0.50
0.10
0.05
Relaxation Time
Q10 = 3.05
u = 20,680 calories
Initial Contraction Time
Q10 = 1.09, u = 2,600 calories
Range used for activation energy (u) and Q10 calculations
40°
23°
TEMP° C
40°
35°
30°
25°
20°
.00320 .00325 .00330 .00335 .00340 .00345
1/TEMP. ABS.

FIG. 4b

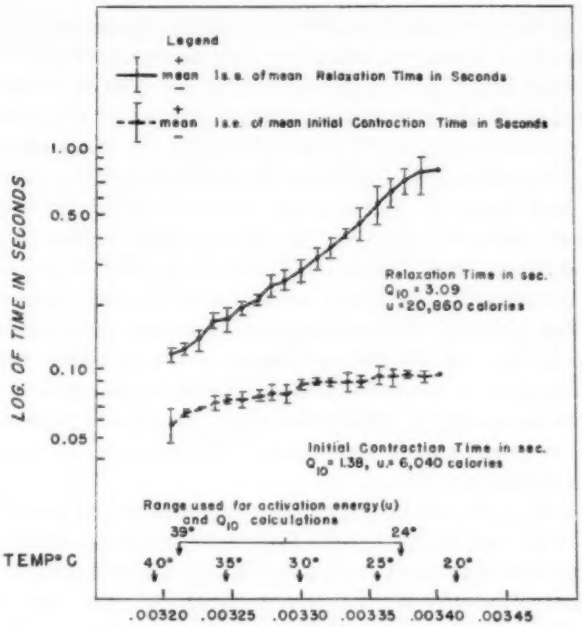

FIG. 4c

FIGS. 4a, 4b, 4c. Temperature dependence of the time requirement for initial contractile force and relaxation in thyroid fed, euthyroid, and athyroid dogs.

The relationship of heart rate to temperature yielded Q10 values (Fig. 8) similar to those observed for oxygen consumption, although there was a departure from linearity over the range studied.

Relation between Metabolic Rate and Duration of Contracted State and Relaxation

It has been demonstrated that there are, in general, similar quantitative variations in myocardial oxygen utilization and in total body oxygen utilization associated with variations in temperature or thyroid concentration (26, 27 28, 29, 30), although a departure from linearity has been noted (29). For this reason and for the general demonstration of the relationship of changes in the rate of oxidative metabolism of the body to the kinetics of contraction and relaxation of the heart, the metabolic rate of the animals at 37°C. was plotted on log scale as a function of the duration of the contracted state, or of the time required for relaxation, similarly plotted on a log scale (Figs. 9 and 10). In all groups of animals, an *inverse* exponential relationship could be shown to exist between these parameters. A decrease in the rate of metabolic activity and thus

of the rate of energy transfer resulted directly in a prolongation of the contracted state and a slowing of relaxation. *The inverse exponential relationship thus demonstrated lends further significance to the high Q10 and activation energy values observed for the duration of the contracted state and for relaxation.*

To emphasize the above point, and to illustrate the characteristics of contraction and relaxation at the extremes of metabolic activity, Figures 11 and 12 are presented. Figure 11 illustrates the curves of contraction and relaxation at extremes of metabolic activity in the intact animal. Figure 12 presents, in idealized graph form, data obtained in 3 thyroid fed animals at a temperature of 40°C. as contrasted to 3 athyroid animals at 22°C. Both figures emphasize a) that the time of initial contraction is least affected by extremes of metabolic activity and b) that the conspicuous feature of low metabolic activity is an extreme prolongation of contraction and a slowing of relaxation. These curves illustrate that the energy of metabolism is not required to maintain the contracted state of muscle and that relaxation is slowed in parallel with a decrease in the rate of metabolic activity.

Analyses of the right and left atrial mean pressures and, in some dogs, of the end diastolic right and left ventricular pressures revealed no significant change over the range of temperatures studied within each group of animals. The mean atrial pressures of the athyroid dogs were significantly higher than those of the euthyroid and thyroid fed group of dogs. This information, coupled with the above data, indicates that *"vis a tergo"* forces are of less consequence in filling of the ventricles during diastole. Rather, the rate and extent of relaxation and consequent filling by *"vis a fronte"* forces is indicated, these forces resulting primarily from active relaxation of ventricular heart muscle (31).

Figures 13, 14a, 14b and 14c summarize the significant alterations in the kinetics of contraction and relaxation produced by variations in thyroid hormone concentration and/or by temperature. Again, it can be seen that at any temperature, initial contraction is least affected by variations in thyroid hormone concentration. The characteristic effects are primarily seen in the contracted state and relaxation. *The rapidity of onset (termination of the contraction) and completion of relaxation is the single kinetic feature of activity in ventricular heart muscle that reflects major variations in metabolic activity induced either by thyroid hormone activity or by temperature.*

Repolarization of Heart Muscle

Variations in the duration of the S-T interval, recorded from an extremity lead, give an estimation of the effect of temperature and thyroid hormone concentration upon repolarization. The S-T interval was found to have a Q10 of 2.3 to 2.48 over the range of 40°C. to 23°C. (Fig. 15). Repolarization of nerve and muscle and the exchange of Na+ and K+ against concentration gradients

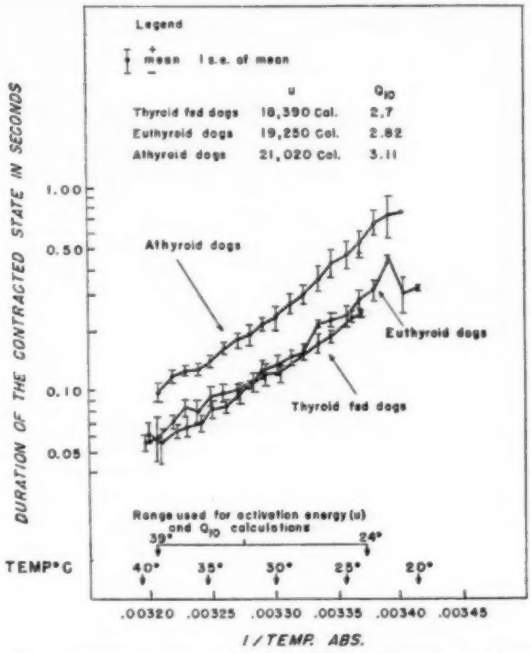

FIG. 5. Effect of temperature upon duration of contracted state of ventricular heart muscle of thyroid fed, euthyroid, and athyroid dogs.

in red blood cells, nerve, and muscle have been demonstrated to be active processes related to the rate of metabolic activity (32 to 30). The above Q10 values for repolarization are in keeping with this fact. The differences in the rate of repolarization of the athyroid, euthyroid, and thyroid fed dogs were not consistently significant over the temperature range studied. The Q10 values for repolarization, though similarly high, differed consistently from those observed for relaxation.

DISCUSSION

Relaxation of heart and striated muscle has been regarded by some investigators as a passive process, produced by internal elastic or external extending forces. As such, the forces producing relaxation of ventricular heart muscle have not seemed sufficiently interesting to most investigators to warrant attention (3, 4, 5, 6, 41).

It has been demonstrated that passive processes such as the diffusion of ions along concentration gradients, changes in viscosity or the rate of viscous flow, ionic interactions between oppositely charged ions or groups, and changes

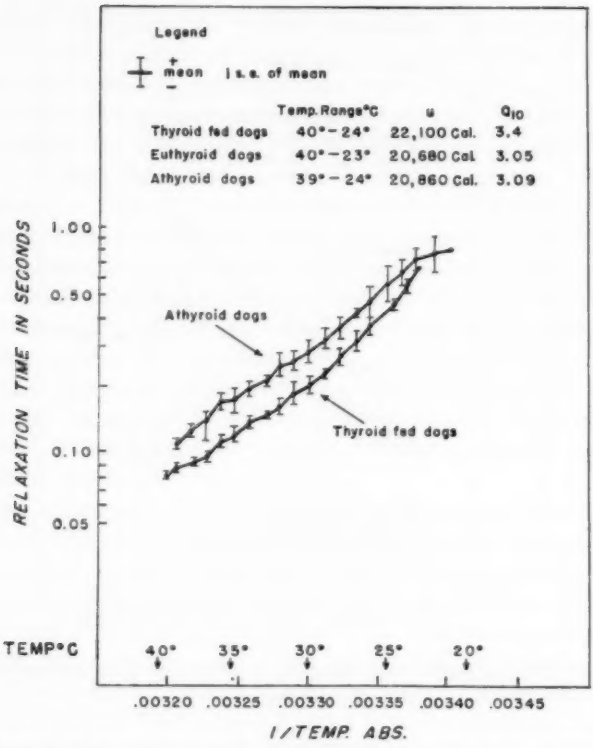

FIG. 6. Effect of temperature upon time required for relaxation of ventricular heart muscle of thyroid fed, euthyroid, and athyroid dogs. (Euthyroid dog values are omitted, because they follow so closely those for the thyroid fed dogs.)

in elastic behaviour, such as stress relaxation, have, in general, Q10 values between 1.1 to 1.3 over the temperature range of 40 to 20°C. (19, 32, 33, 34, 35, 36, 37, 42, 43, 44). In general, the processes outlined have rarely been found to have Q10 values exceeding 2.0. The physical processes outlined above have correspondingly low activation energies, ranging in general between 2,000 to 6,000 cal./mole. In contrast, active chemical reactions, implying electron or chemical bond energy transfer between atoms or molecules, in general, follow van't Hoff's rule with Q10 values of 2.0 to 3.0 and activation energy values which vary from 12,000 to 20,000 cal./mole. On the other hand, numerous examples of redox reactions with Q10 values of 1.3 to 1.4 and (u) values in the range of 2,000 to 6,000 cal./mole have been observed (22, 23).

With the above in mind, the low Q10 values observed for the force/time impulse of contraction noted above do not eliminate the possibility of an

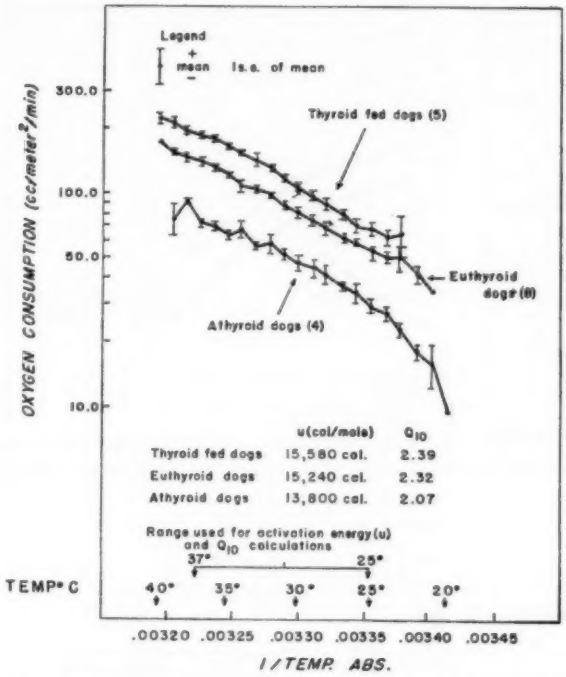

FIG. 7. Effect of temperature upon oxygen consumption of thyroid fed, euthyroid and athyroid dogs.

enzymatically catalyzed chemical reaction as responsible for initial contraction. However, the Q10 values for the time, force, or rate of initial contraction are most characteristic of a physical process involving ionic interaction, diffusion, or viscous flow. The observed Q10s for contraction are thoroughly in keeping with the theories of Szent-György, Morales, and Ramsey concerning the nature of ionic or entropic forces responsible for contraction of heart muscle (1, 2, 7, 8, 10, 11, 12). Szent-György has demonstrated that muscle in the relaxed state is regarded as essential for the high affinity of dissociated actin and myosin for both water dipoles and for univalent and divalent cations. Contraction from this point of view involves a depolarization and discharge of the proteins, loss of water, and precipitation or "superprecipitation" of actin and myosin. As such contraction would be in essence an entropic process involving an ionic interaction between oppositely charged groups of actin and myosin and possibly involving ATP adsorption as outlined both by Szent-György and Morales. As such, the impulse of initial contraction should follow closely that of an ionic interaction and should be impeded by changes in the viscosity of water and

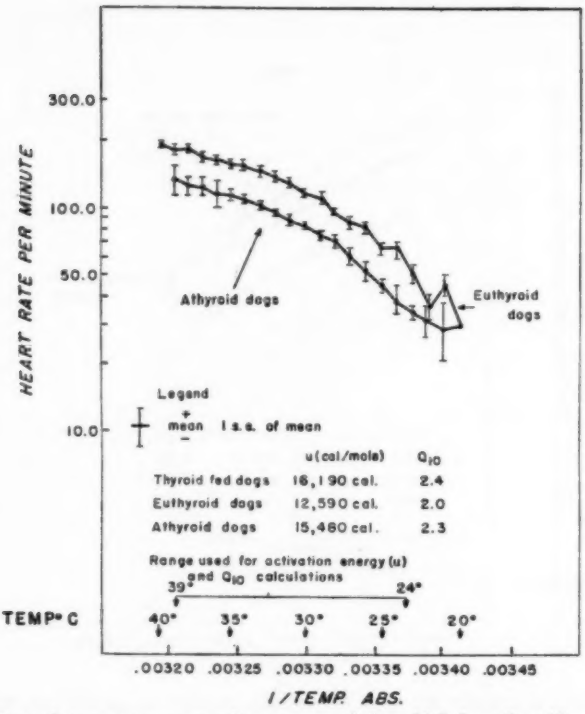

FIG. 8. Effect of temperature upon heart rates of thyroid fed, euthyroid, and athyroid dogs. (Thyroid fed dog values are not plotted, but Q10 and activation energy values are shown.)

passive diffusion of ions along concentration gradients with retarded loss from the proteins at lowered temperatures. The slowing of initial contraction with a lowering of temperature, if precipitation of actin and myosin or an association of their side groups involves loss of water dipoles, should agree well with the experimental data presented above. Over the range of 40°C. to 30°C., the viscosity of water increases with a Q10 of 1.22. Between 30° to 20°C. the change in the viscosity of water has a Q10 of 1.25.

In marked contrast, relaxation as a rate process was found to possess none of the kinetic characteristics expected of a passive process. The calculated Q10 values for the duration of the contracted state and for relaxation do not fall in the range of passive processes, are characteristic of chemical reactions involving bond energy or electron transfer, and suggest that in order for relaxation to proceed, the average energy of the reacting molecules must equal or exceed the observed values of 18,720 to 22,100 cal./mole. These activation energies and Q10 values are in excellent agreement with such known active processes,

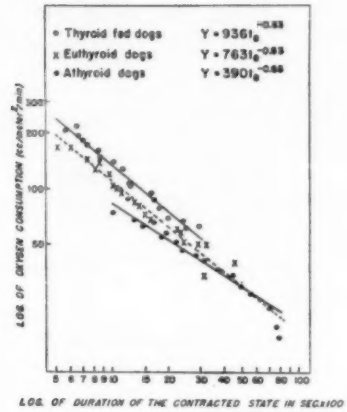

FIG. 9. Relationship between oxygen consumption, plotted on log scale (Ordinate), and the duration of the contracted state, similarly plotted on log scale (Abscissa).

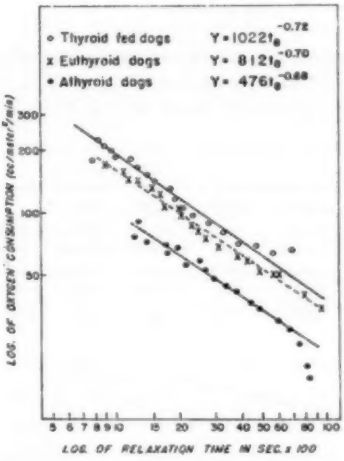

FIG. 10. Relationship between oxygen consumption, plotted on log scale (Ordinate), and the time required for relaxation, plotted on log scale (Abscissa).

such as Na+ efflux and K+ uptake against concentration gradients by nerve, muscle and red blood cells (32–40), renal tubular excretion of para amino hippurate (45, 46), and rate of metabolism and activity of intact and isolated tissues (18, 19, 20, 21). The inverse exponential relationship between the duration of the contracted state or the rate of relaxation and the total rate of oxidative metabolism is a striking indication of the metabolically active nature of relaxation.

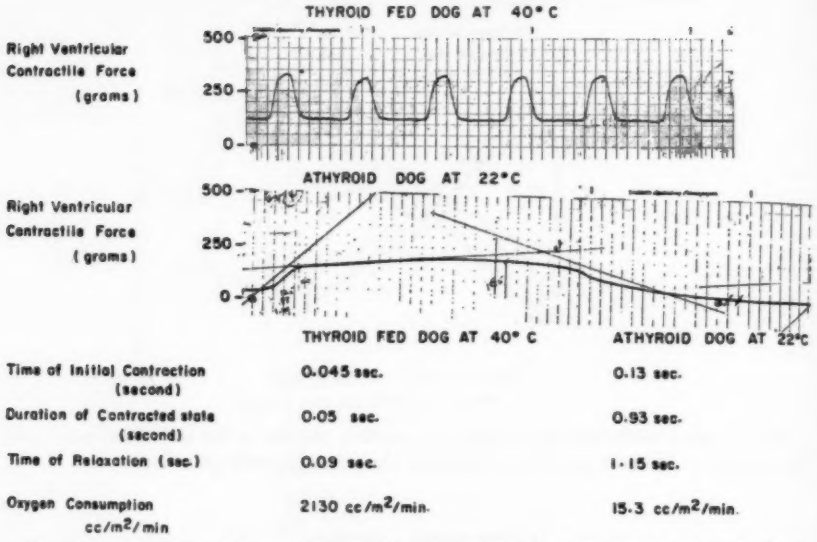

FIG. 11. Comparison of curves of isometric contraction and relaxation of ventricular heart muscle from thyroid fed dog at 40°C. and athyroid dog at 22°C. Paper speed: 1 mm. = 0.01 seconds. (O_2 consumption of thyroid fed dog at 40°C. is 213.0 cc./M^2/min.).

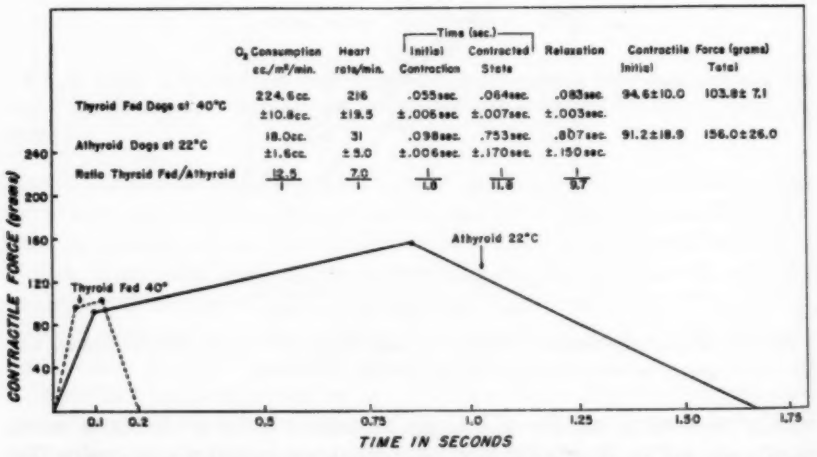

FIG. 12. Schematic comparison of times of contraction and relaxation in three thyroid fed dogs at 40°C and in three athyroid dogs at 22°C. Times obtained from means of data.

There is an excellent correlation in principle between the results obtained above and the results obtained by Lambert, *et al.* (47) in a study of isometric contraction and relaxation of human striated muscle. Lambert and his associates studied the Achilles tendon reflex in hyperthyroid, euthyroid, and in

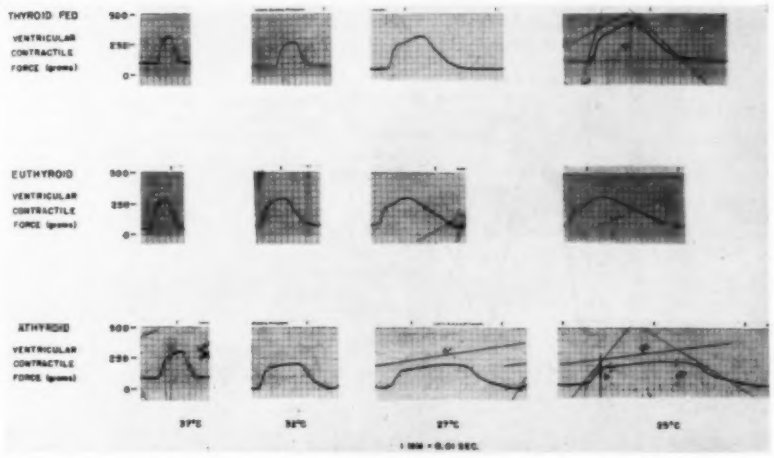

FIG. 13. Recordings of contraction and relaxation in thyroid fed, athyroid, and euthyroid dogs at 37°, 32°, 27°, and 25°C. Paper speed 1 mm. = 0.01 seconds.

athyroid individuals. They demonstrated that variations in thyroid activity resulted primarily in a prolongation of the contracted state and a slowing of relaxation. They found, in agreement with our results, that there was a correlation between the basal metabolic rate and the rate of relaxation, the lowest BMR being associated with the slowest rate of relaxation. It was noted that the stimulus-depolarization time, depolarization-contraction time, (and from their curve) the time of initial contraction did not vary as a function of the metabolic rate. Studies of the effect of lowered extremity temperature similarly were shown to prolong the duration of the contracted state and slow relaxation. We have carried out studies in the frog and dog gastrocnemius that corroborate their results.

The results appear to justify the concepts relating relaxation to an increase in the potential energy of the contractile protein system with dissociation or elongation of actin and myosin resulting from Coulomb repulsive forces at charged points of the protein molecules. From a thermodynamic analysis Szent-György and Varga (7, 8) indicated that actin and moysin were extended and dissociated by chemical bond energy or electron transfer. Our results agree well with this approach.

The active nature of relaxation noted above receives further support from the following observations:

1. Active extension of the single muscle fiber has been demonstrated (48), as well as ventricular suction in amphibian and mammalian hearts (31, 49).

2. The rate and *extent* of relaxation are not passive constants but are fundamentally affected by drugs altering the "contractility" of heart muscle. It has been demonstrated that drugs or hormones exerting a positive inotropic effect

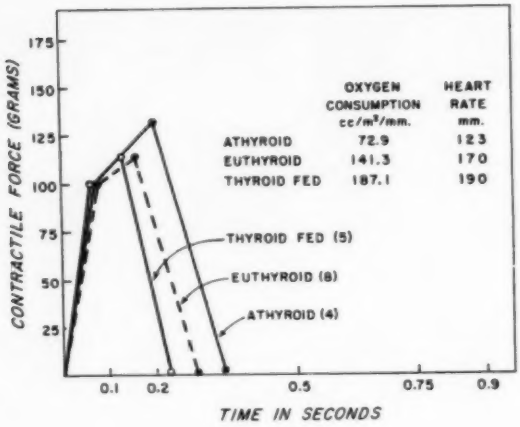

FIG. 14a

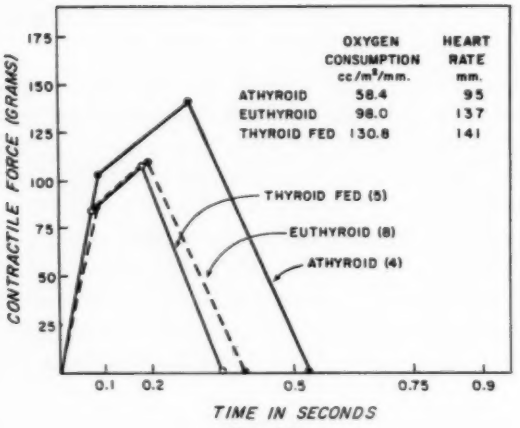

FIG. 14b

increase rate and extent of relaxation, thereby decreasing the impedance to ventricular filling (31, 50, 51, 52). Drugs or hormones with a negative inotropic effect decrease the rate and extent of relaxation and increase filling impedance. Similar data have been obtained by Rushmer (53, 54) who found ventricular diameter and circumference during relaxation to be increased by epinephrine and norepinephrine and decreased by a barbiturate.

3. Fatigue and ischemia have been demonstrated to profoundly slow relaxation and to result in incomplete relaxation (2, 55).

4. Under circumstances where there is a profound influence upon rates of phosphorylation and electron transport there is a slowing of relaxation coupled with an increase in the tension developed by contracting muscle. Edwards

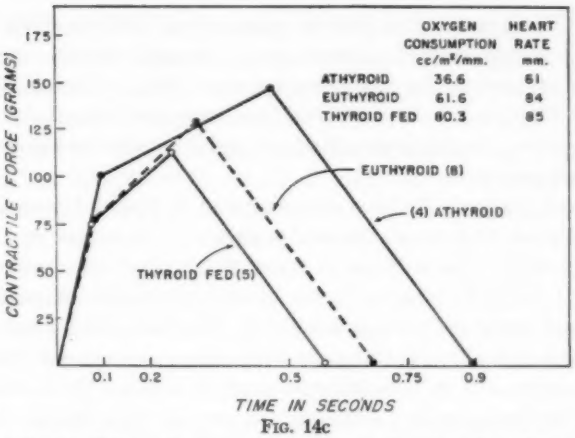

FIG. 14c

FIGS. 14a, 14b, 14c. Schematic comparison of times of contraction, the contracted state, and relaxation of thyroid fed, athyroid, and euthyroid dogs at 37°, 32°, and 27°C. Times obtained from means of data.

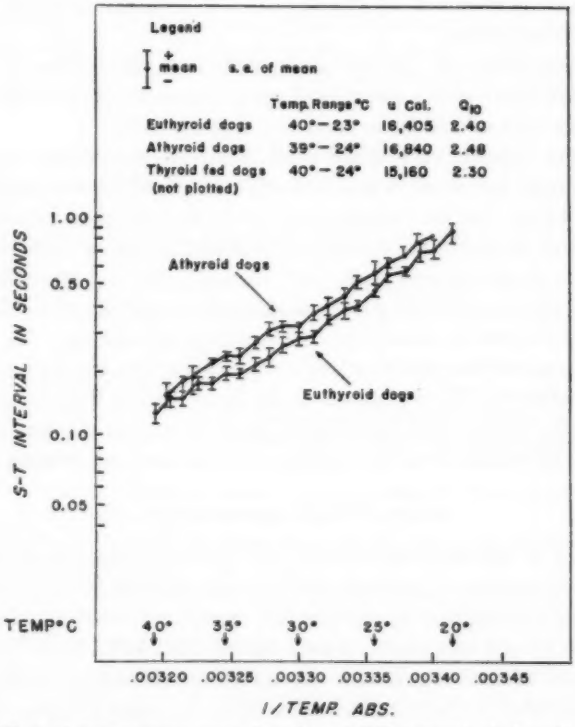

FIG. 15. Effect of temperature upon duration of S-T interval of thyroid fed, athyroid, and euthyroid dogs.

and Brown (56) demonstrated that at pressures of 2,000 to 6,000 P.S.I. the isolated turtle auricle showed an increase in contractile tension and a slowing of the rate of relaxation, the rate of contraction being unaffected. Separately, Deuticke and Harren (57) demonstrated that, in pressed muscle juice, the rate of phosphorylation, dephosphorylation, and redox reaction are slowed at pressures of 800 atmospheres.

5. Jobsis and Chance (58) have demonstrated, including calculations of the time of diffusion of ADP from unfavorable sites, that no release of ADP can be detected until contraction is 80 per cent completed. They demonstrated simultaneously that electron transport in the cytochrome chain commenced late in contraction and continued through relaxation. The close coincidence of possible ATP hydrolysis and electron transport with events occurring at the termination of contraction is of extreme interest in relation to our demonstration that the duration of the contracted state and the rate of relaxation are linked with temperature changes with kinetic characteristic expected of ATP hydrolysis and associated electron transport. Further work is necessary along this line. This relationship is supported by the observations of Guthe (59) that the activation energy for hydrolysis of ATP by Myosin B in an isolated septum is approximately 31,000 cal./mole.

6. The prolongation of the contracted state and the slowing of relaxation showed in data above with a lowering of temperature are in agreement with the work of Berne (60) and of Reissmann and Kapoor (61).

Furthermore, Starling's law of the heart, which has stated that the release of mechanical energy by muscle in systole is related to the diastolic fiber length, has for years indicated that the release of energy by the system is a function of the potential energy stored in the system. This potential energy definitely has two components: a metabolic and a physical. Szent-György (7) has indicated that the filling pressure or extending force of either increased fiber lengths or initial tension may be stored in muscle as chemical potential energy. The work outlined above in no way negates Starling's original concept, but adds information to the effect that the filling pressure of the heart during the relaxation phase plays a complementary role to chemical bond energy or electron transfer in determining the potential energy available for contraction or work.

SUMMARY AND CONCLUSIONS

The kinetics of isometric contraction and relaxation have been observed in intact athyroid, euthyroid, and thyroid fed dogs utilizing Walton strain gauge arches sutured to segments of right or left ventricular heart muscle. The animal's heart and body temperatures were varied from 42°C. to 17°C. Isometric contraction and relaxation of muscle displayed four distinct phases for analysis: (1) Initial contraction characterized by a rapid and near maximal increase in

tension (2) Contracted state during which a further slow increase in tension may occur (3) Relaxation (4) Relaxed state. The temperature coefficients (Q10) and Arrhenius activation energy (u) values were calculated for the duration and tension of the above states, for the S-T repolarization interval, for the heart rate, and for the basal metabolic rate or oxygen consumption. The Q10 values for the duration of the phase of initial contraction and for the tension developed varied from 1.09 to 1.38 (u values of 2,600 calories to 6,040 calories per mole). In contrast, the duration of the contracted state and the time required for isometric relaxation had Q10 values varying from 2.7 to 3.4 (u values of 18,390 to 22,100 cal./mole). An inverse, exponential relationship existed between the metabolic rate and the duration of the contracted state or the time required for relaxation. Contraction possesses the characteristics of a physical process involving ionic interaction, diffusion, or viscous flow. Relaxation has the kinetic characteristics of an enzymatic reaction associated with and dependent upon chemical bond energy or electron transfer. Relaxation of ventricular heart muscle thus has the characteristics of an active metabolic process, its rate related directly to thyroid hormone concentration and temperature.

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HYPER-IMMUNIZATION OF MAN[1]

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Repeated immunization of man with antigenic materials from a variety of pathogenic microorganisms has become an accepted measure to prevent infection. Such immunization can produce hypersensitivity to microbial antigens, and reinoculation of antigen or vaccine into the hypersensitive person may elicit acute local and systemic reactions of varying severity. Few other untoward effects from *repeated* immunization of man, however, have been clearly defined.

Intensive immunization of experimental animals has been shown (1–20) to produce delayed adverse consequences, i.e. amyloid deposition, arteritis, etc., but similar observations have not been conclusively reported in the human being, possibly related to the fact that far greater quantities of antigen are given to the experimental animal than would ordinarily be given to man.

We have had an opportunity to study a number of men who have been immunized prophylactically with a variety of bacterial, rickettsial, and viral antigens because of their employment in laboratories investigating pathogenic microorganisms. These men had received immunizations far in excess of that usually administered. To evaluate the effect of hyper-immunization in man these subjects were studied, and the results of clinical and laboratory examination are presented.

MATERIAL AND METHODS

A group of 99 Caucasian males between the ages of 28 and 65 years (Table I-A) was studied.

Diseases against which the personnel were immunized included botulism, brucellosis, tularemia, anthrax, diphtheria, Rocky Mountain spotted fever, Q fever, plague, typhus, psittacosis, smallpox, Eastern, Western, and Venezuelan equine encephalitis. The antigens used for immunization varied considerably in type, method of preparation, and concentration, so that a complete listing here is not practical. They have, however, been described elsewhere (21–27)[2], and consisted of killed bacterial vaccines, purified bacterial fractions, toxoids, and chicken egg prepared virus vaccines. The duration of the immunization period varied from 8 to 13 years during the time from 1944 to 1956 (Table I-B). The total amount of all antigens administered to the men (Table I-C) ranged from 35.8 to 74.4 ml., and varied in

[1] Supported by contract DA-18-064-404-CML-100 with the Army Chemical Corps, Fort Detrick, Maryland.

[2] Rocky Mountain spotted fever, Q fever, and Typhus vaccines, Lederle Laboratories, New York, N. Y. Plague vaccine, Cutter Laboratories, Berkeley, Cal. Smallpox vaccine, Parke, Davis and Co., Detroit, Mich.

TABLE I

Basic Data

A. Age

Range: 28 to 65 years
Mean: 40.1 years
Distribution:

age (yrs.)	(no. subjects)
28–30	4
31–35	27
36–40	26
41–45	20
46–50	9
51–55	7
56–60	3
61–65	3
Total	99

B. Duration of Immunization

Range: 8 to 13 years
Mean: 10.4 years
Distribution:

time (yrs.)	no. subjects
8	5
9	21
10	17
11	43
12	9
13	4
Total	99

C. Antigen Dose

Range: 35. to 74. ml.
Mean: 52.8 ml.
Distribution:

ml. antigen	no. subjects
35–40	6
41–45	20
46–50	21
51–55	15
56–60	19
61–65	12
66–70	5
71–75	1
Total	99

D. Skin Tests

Range: 9 to 44 per subject
Mean: 20.1 per subject
Distribution:

tests per subject	no. subjects
6–10	5
11–15	25
16–20	22
21–25	26
26–30	16
31–35	4
36–40	0
41–45	1
Total	99

E. Immunization Reactions

Range: 0 to 16 per subject
Mean: 2.8 per subject
Distribution:

reactions per subject	no. subjects
0	18
1–5	68
6–10	10
11–15	2
16	1
Total	99

each case with the duration of the immunization period. All subjects received immunization with antigens of botulism, tularemia, Rocky Mountain spotted fever, Q fever, typhus, plague, psittacosis, and the viral encephalitides. Thirty-seven were immunized against brucellosis, 95 against smallpox, 25 (all of whom had initially positive Schick tests) against

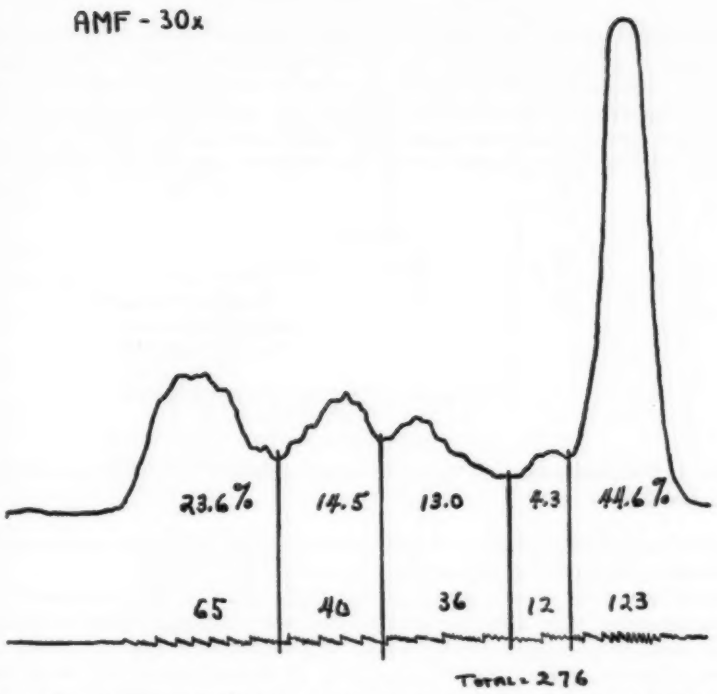

FIG. 1. Normal serum electrophoretic pattern demonstrating method of determining per cent of each protein fraction from electrophoretic curve integrated according to the area under each peak. "276" is representative of the area under the curve. Each protein fraction is expressed in per cent of total protein.

diphtheria, and 28 against anthrax. With each antigen, an initial series of immunizing injections was given, followed by "booster" injections every 6 to 12 months. In addition, subjects underwent frequent skin testing with the antigens of tuberculosis (Purified Protein Derivative), diphtheria (Schick and Maloney), brucellosis (Brucellergin), tularemia (Foshay), histoplasmosis, blastomycosis, coccidioidomycosis and glanders (28).[3] The average subject received about 20 such skin tests during the period of immunization (Table I-D). Reactions were frequent, ranging from none to 16 per subject (Table I-E). Serological titers to tularemia (agglutination) were performed frequently on all subjects. Titers to brucellosis (agglutination), Rocky Mountain spotted fever (complement fixation) (CF), Q fever (CF), typhus (CF), and psittacosis (CF) were performed less frequently and were not analyzed for this reason.

Ninety-three of the 99 men were evaluated by complete medical history and physical examination. In addition, each person's out-patient and hospital record for the period of

[3] Tularemia—1:1000 dilution of Foshay vaccine; Purified Protein Derivative Tuberculin, and Brucellergin, Sharpe and Dohme, Philadelphia, Pa., Histoplasmin and Blastomycin, Parke, Davis and Co., Detroit, Mich., Coccidioidin and Diphtheria toxin, Cutter Laboratories, Berkeley, Cal.

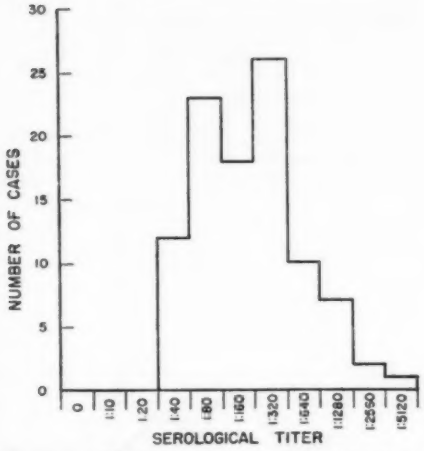

FIG. 2. Distribution curve of highest tularemia agglutinin titer obtained in the hyperimmunized persons.

immunization was reviewed. Since the subjects were followed in one clinic, reliable records of all illnesses occurring during the period of immunization were obtained.

Eighty-nine of the 99 men were evaluated by the following clinical laboratory tests: electrocardiogram, chest x-ray, hematocrit (Wintrobe), total and differential leukocyte counts, blood urea nitrogen (BUN), 2-hour phenolsulphthalein excretion (PSP), blood cholesterol, serum alkaline phosphatase activity (Bodansky units), total serum protein (TSP), serum albumin-globulin ratio, serum cephalin-cholesterol flocculation, serum thymol turbidity, and bromsulphalein (BSP) retention 45 minutes after intravenous injection of 5 mg. per kg. of the dye.

Serum for paper electrophoresis was obtained by centrifugation of venous blood after clotting and stored at 4°C. Duplicate specimens of 0.01 ml. of each serum were subjected to electrophoresis for 16 hours at 10 ma. in a Spinco Durrum-type electrophoresis cell (29) on 3 by 32 cm. Whatmann No. 3 filter paper strips, using 0.075 M veronal-veronal buffer at pH 8.6. After electrophoresis, the protein was coagulated and fixed to the paper by heating in an oven at 120°C. for 30 minutes. The protein was stained by immersing the paper strip for 8 hours in a solution containing 0.01 per cent bromphenol blue and 3.1 per cent zinc sulfate. The strips were then washed twice for 6 minutes in 5 per cent acetic acid, and fixed for 6 minutes in a 0.9 per cent solution of sodium acetate in 5 per cent acetic acid. After drying for 20 minutes at 120°C., the strips were exposed for 2 minutes to ammonia vapor. The stained strips were scanned photo-electrically in a Spinco model R Analytrol[4] for direct writing of the electrophoretic curve and automatic integration to determine the area representative of each of the 5 major protein components. Figure 1 shows a normal electrophoretic pattern with intersects drawn at the lowest point between each protein fraction. The area of the curve between the intersects was used in calculating the per cent of each protein constituent.

The total nitrogen of each serum sample was measured in duplicate by the micro-Kjeldahl method. Total *serum* nitrogen rather than total *serum protein nitrogen* was estimated. This

[4] Beckman Instruments Inc., Belmont, Cal.

choice was made because of the relative simplicity of total serum nitrogen determinations and all subjects had normal serum non-protein nitrogen levels. The fraction of total serum nitrogen represented by non-protein substances was negligible. Quantitative determination of albumin and alpha-1, alpha-2, beta, and gamma globulin expressed in mg. nitrogen per ml. of serum, was calculated from the fraction per cent obtained from electrophoresis and the total serum nitrogen. The nitrogen value for each protein component was used for statistical analysis.

TABLE II

History of Illness

A. *Probably Occupational*	
Tularemia, symptomatic	1
"Chronic brucellosis"	1
Q fever	1
Undefined fevers	9
B. *Not Occupational*	
Chronic sinusitis	10
Renal disease	10
Calculi	4
Acute pyelitis	4
Glomerulonephritis	1
Renal cyst	1
Allergy (hay fever, asthma, drug reactions)	9
Duodenal ulcer	7
Hepatitis	5
Arthritis	5
Hypertension	4
Coronary occlusion and/or angina pectoris	4
Albuminuria (orthostatic)	3
Diabetes mellitus	2
Acute rheumatic fever	2
Lung abscess	1
Osteomyelitis	1
Gout (?)	1
Cushing's syndrome (probable)	1
Colloid goitre	1
Leriche syndrome (occlusive arterial disease)	1

TABLE III

Differential Leukocyte Count

1. Lymphocytosis (> 36 per cent)

per cent	no. subjects
36–40	25
41–44	16
45–50	2
51–55	2
56–60	3
61–65	1
Total	49

For comparative analysis, the sera of 44 normal medical students, house-officers, nurses, and laboratory personnel, ages 18 to 50 years, were studied by electrophoresis in the same way as the sera from the hyper-immunized subjects. The control group selected was, in many aspects, not an adequate control for the experimental group. We were not able, however, to assemble a group of identical age, sex, and occupational exposure who had not received intensive immunization.

RESULTS

Serological Titers: Figure 2 shows a distribution curve of the highest tularemia titer obtained on each immunized subject during the period of study. No one failed to develop a rise in titer during his series of injections. When the serologic titers of the group were compared with those of persons of comparable age and duration of immunization who were immunized against tularemia only, there appeared to be no difference in the distribution of titers obtained in the two groups. This suggests that administration of multiple immunizing agents probably did not interfere with development of an adequate antibody response to this antigen.

Clinical Evaluation: As a group the men were not ill. Of the 93 patients queried, 55 had some illness in the past which might possibly alter one or more of the laboratory tests used in the study. These illnesses probably appeared no more frequently than might be expected in other individuals of comparable age, sex, and employment. There were a few instances of occupational infection. One patient had recovered from pulmonary tularemia, one may have had brucellosis, and another man had recovered from Q fever. Nine patients had short febrile illnesses of undetermined etiology without evidence of occupational disease and were diagnosed as having upper respiratory in-

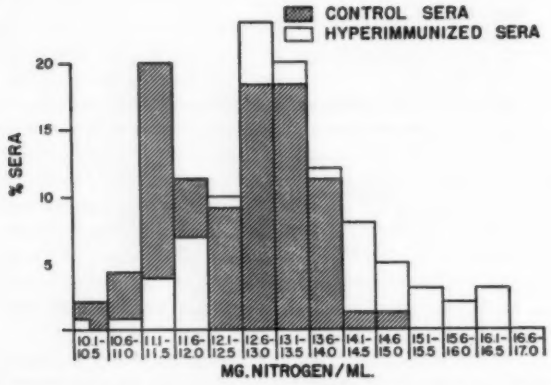

FIG. 3. Distribution of total serum nitrogen levels in 99 hyper-immunized men (99 sera) and 44 normal persons (44 sera), determined by electrophoresis.

fection. No illness was detected by anamnesis which might have been directly attributable to immunization. It is impossible, of course, to eliminate the possibility that some of these men had sub-clinical occupational illnesses. There were 5 instances in which an unexpected rise in tularemia titer occurred, and these were diagnosed as "serological tularemia". There were no similar rises in the serological titers for other diseases to which these men were exposed. One can assume, therefore, that the occurrence of such sub-clinical infection was probably uncommon.

The occurrence of hypertension and valvular murmurs among the immunized group was no different than that expected in any other population. Hepatomegaly was probably accounted for in 2 patients by a recent history of tularemia and brucellosis, respectively. In 5 other subjects there was no ready

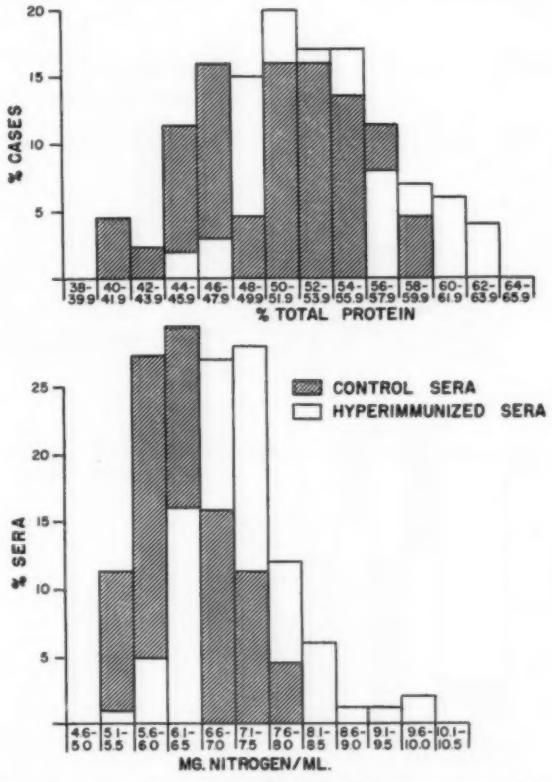

FIG. 4. Distribution of serum albumin levels, expressed as per cent of total protein and as mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

explanation for enlargement of the liver. Alcoholism was not diagnosed in any of the individuals. Macroglossia was detected in one patient.

Clinical Laboratory Evaluation: The hematocrit was determined in 91 subjects and the only abnormality was polycythemia in a patient with probable Cushing's syndrome. Twenty of the men examined had leukocytosis (10–16,000 per ml.), and 2 had leukopenia (4–4900 per ml.). Three subjects had a blood monocyte count in excess of 10 per cent. Seventeen patients had eosinophilia. In three of these there was a history of an allergic disorder such as hay fever. Forty-nine men had lymphocytosis (Table III). Of these, 7 had a history of an allergic illness, 4 of arthritis, three of non-specific febrile illnesses in the remote past, 3 of serological tularemia, one of clinical tularemia, and one each of Q fever, paratyphoid fever, and long standing myalgia of undetermined etiology. With the exception of one instance of serologic tularemia, the allergic

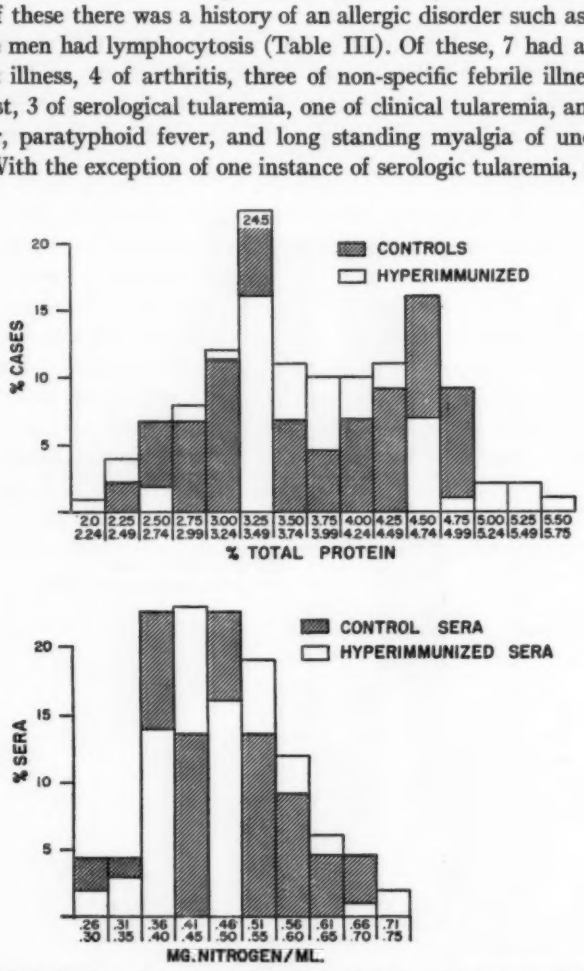

FIG. 5. Distribution of serum alpha-1 globulin levels, expressed as per cent of total protein and as mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

disorders and the chronic myalgia, all of these illnesses had occurred many months prior to the laboratory evaluation. It is unlikely that such remote illnesses could have had an effect upon the lymphocyte count at the time of the study.

Chest x-ray showed a few abnormalities among 89 men examined, including punctate calcification in 2, pleural thickening in one and hilar adenopathy in another. These abnormalities seemed to bear no relationship to immunization.

Proteinuria of varying degree was detected in about one-third of the patients but in only 2 subjects was this greater than one-plus, and probably could be explained by the presence of hypertension, history of glomerulonephritis, arteriosclerotic disease, or may have been accounted for on a functional basis. There was no reduction in phenolsulphthalein excretion which could not be readily explained.

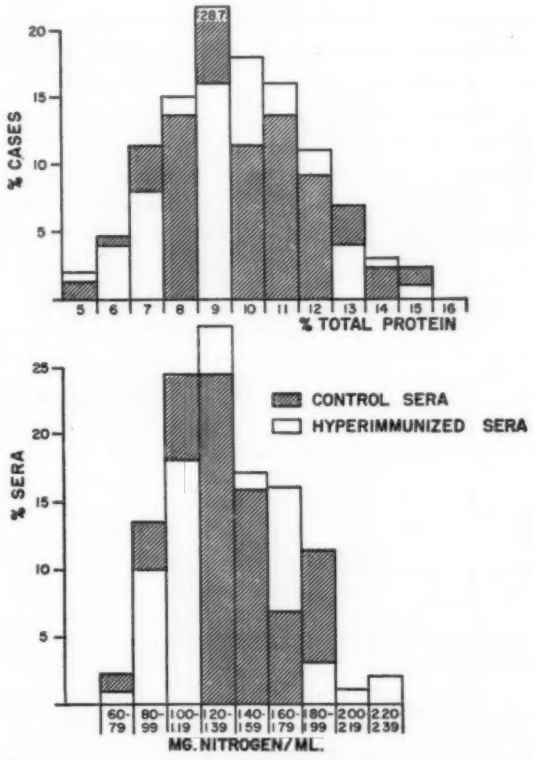

FIG. 6. Distribution of serum alpha-2 globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

In no patient was the blood cholesterol or alkaline phosphatase level significantly abnormal. Twenty-six men had a positive serum cephalin-flocculation test (1 or 2 plus) which was unexplained. In 7 persons the serum thymol turbidity was above 5 units (5–11) for an unexplained reason, and, 7 patients had a slightly impaired bromsulfalein excretion (5–10 per cent). The total serum proteins (grams per cent) and albumin-globulin ratios were not particularly abnormal in any individual.

In all, 53 subjects were found to have one or more abnormalities of liver function test. Excluded from this number are 25 persons with a past history of either allergy, arthritis, non-specific fever, tularemia, possible brucellosis, rheumatic heart disease, Q fever, probable Cushing's syndrome, osteomyelitis, or undiagnosed myalgia, all of whom had at least one abnormality of these laboratory tests.

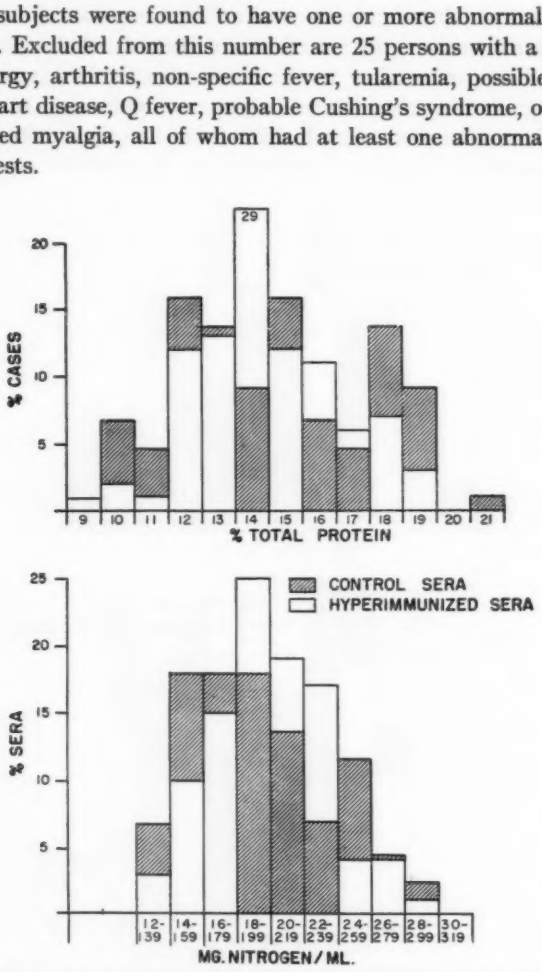

FIG. 7. Distribution of serum beta globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

An abnormal electrocardiogram seen in 4 patients was compatible with a history of coronary occlusion, rheumatic heart disease or hypertension. One unexplained right bundle branch block was found.

Serum Electrophoretic Studies: The mean total serum nitrogen and albumin nitrogen for the immunized group, estimated by electrophoresis, was significantly higher than that of the control group. There was no significant difference between the means of any of the other protein fractions in the test and control groups. Distribution curves for the various protein fraction values, calculated as per cent of the total and mg. nitrogen, are plotted in Figures 3–7. A tabular analysis is presented in Table IV. Gamma, alpha-1, alpha-2, and beta globulins were not significantly different in the immunized and control groups. The distribution curves of albumin and total serum nitrogen in the immunized group, however, were skewed toward the high levels. Eight subjects had total serum nitrogen values significantly higher than normal which could not be explained by any underlying disease. Similarly, 4 subjects had elevated total serum albumin. These 2 abnormalities occurred concurrently in 2 subjects.

There was one type of distinct qualitative abnormality of electrophoretic pattern which occurred frequently in the immunized subjects. Twenty-three of the 99 patients had serum electrophoretic patterns with a marked disturbance in the alpha-2 globulin region with poor separation of the alpha-2 and beta globulin fractions. This abnormality is illustrated in Figure 9. Although this pattern occurred in the hyper-immunized group with a frequency of 23 per

TABLE IV

Statistical Analysis of Serum Electrophoretic Fractions

Fraction	Group	mg. Nitrogen/ml. serum				Per cent, total protein			
		Range	Mean	±2 SD	SD	Range	Mean	±2 SD	SD
Gamma Globulin	Control	1.6–3.4	2.5	±1.05	0.52	14.1–26.1	20.2	±3.72	3.36
	Test	1.4–3.9	2.4	±0.98	0.49	11.2–27.0	17.7	±5.12	2.56
Beta Globulin	Control	1.2–2.8	1.9	±0.84	0.42	10.1–19.9	15.2	±5.64	2.82
	Test	1.1–3.0	2.0	±0.69	0.34	10.5–19.9	14.9	±4.54	2.27
Alpha-2 Globulin	Control	0.6–1.9	1.3	±0.64	0.32	5.3–15.8	10.1	±4.54	2.27
	Test	0.7–2.3	1.4	±0.59	0.30	5.5–15.6	10.2	±4.04	2.02
Alpha-1 Globulin	Control	0.27–0.68	0.47	±0.20	0.10	2.4–4.9	3.8	±1.49	0.75
	Test	0.28–0.73	0.49	±0.17	0.09	2.1–5.6	3.7	±1.45	0.73
Albumin	Control	5.3–7.9	6.3	±1.27	0.64	40.4–59.1	50.9	±9.90	4.85
	Test	5.4–9.9	7.1	±1.65	0.83	45.3–63.7	53.6	±13.2	6.61
Tot. Serum N	Control	10.3–14.6	12.5	±2.06	1.03	—	—	—	—
	Test	10.2–16.5	13.3	±2.34	1.17	—	—	—	—

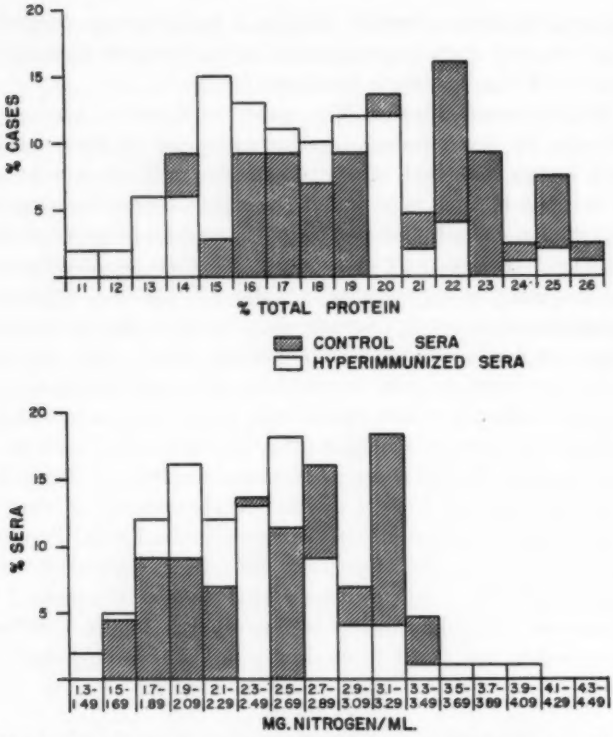

FIG. 8. Distribution of serum gamma globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

cent, it was not seen at all in the control series. Of the 23 patients with this electrophoretic abnormality, 19 were available for clinical study. The ages, duration of immunizations, number of skin tests, and frequency of immunization reactions in this group were identical to those of the immunized group as a whole as well as to the 76 subjects with normal electrophoretic patterns. There seemed to be no relation between the occurrence of this abnormality and the amount of any one antigen administered. When one compares the incidence of the various unexplained laboratory abnormalities in this group with those in the remainder of the immunized men, several differences are apparent. In the group with abnormal electrophoretic patterns, the incidence of hepatomegaly, lymphocytosis, proteinuria, and positive serum cephalin-flocculation are considerably higher. Of these 23 individuals, 3 gave histories of probable occupational infection (tularemia, brucellosis, fever of unknown origin) 7 others had arthritis, hay fever, osteomyelitis, or rheumatic heart disease.

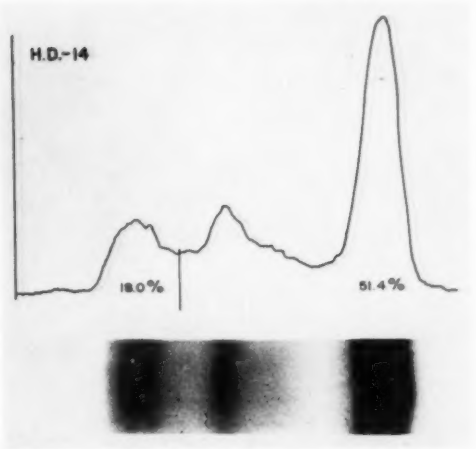

FIG. 9. Abnormal serum electrophoretic pattern observed in 23 per cent of hyper-immunized men, showing poor separation of alpha-2 and beta globulin.

DISCUSSION

In this study of hyper-immunized men, we have found no instance of illness which might be attributed to immunization; nor was there any suggestion of interference with serological response to antigenic stimulus.

The electrophoretic abnormality appears to be the most striking deviation from normal found in these intensively immunized persons. This was characterized by poor separation of the alpha-2 and beta globulin. Similar abnormalities of electrophoretic pattern have been reported to occur in several situations, including the nephrotic syndrome (29–32, 37, 38), disseminated lupus erythematosus (33), and amyloidosis (34–36). Mackay and Volweiler (37) have reported a patient with supposed Kimmelstiel-Wilson syndrome who also had poor separation of alpha-2 and beta globulin on serum electrophoresis. Kuhns (38) has shown that 9 to 19 days following a "booster" injection of diphtheria toxoid, several patients with high "skin-sensitizing" antibody titers demonstrated this same peculiar merging of the alpha-2 and beta globulin. He also found this pattern in one patient with eosinophilia following a penicillin injection and in another with pollen hay fever.

In our series of 44 normal persons the abnormality of electrophoretic pattern was not observed. We did not see a similar abnormality in the patterns of an additional 100 normal persons (medical students) kindly lent to us by Dr. Gerald Klatskin.[5] In reviewing 1000 serum electrophoretic patterns done consecutively on hospitalized patients (Dr. Gerald Klatskin), we found 6 patterns which showed some merging of alpha-2 and beta globulins. These

[5] Yale University School of Medicine, New Haven Hospital, New Haven, Conn.

patients had the following diagnoses: multiple myeloma, probable demyelinizing disease of the brain-stem, pneumococcal pneumonia with septicemia, rheumatoid arthritis, and hypertension, exfoliative dermatitis, and cholelithiasis, diverticulosis, and osteoarthritis.

In view of the low incidence of the serum electrophoretic abnormality in normal subjects (0 per cent) and in hospitalized patients (0.6 per cent), and in view of the high incidence of this abnormality in the hyper-immunized men it seems reasonable to conclude that the protein abnormality may be related to the intensive immunization.

The high incidence of lymphocytosis among the hyper-immunized men (54 per cent), and particularly in those persons with abnormal serum electrophoretic patterns (68 per cent), cannot be explained by any of the clinical observations. The possibility that the immunizations might be responsible for this finding appears likely. Intense antigenic challenge may cause lymphatic hyperplasia and could explain the leukocyte abnormality, but the significance of lymphocytosis in this circumstance is not clear.

Some abnormality of liver "function" tests was present by laboratory examination in over half (59.5 per cent) of the 89 men studied. Only the abnormalities of serum cephalin-flocculation, total serum nitrogen and serum albumin nitrogen seem of possible significance. The occurrence of similar abnormalities among normal subjects, however, makes it impossible to interpret these findings in the immunized patients.

Two findings in the hyper-immunized patients, are not explained on any common basis other than the intense immunization which they received. These include: (1) a peculiar abnormality of the electrophoretic pattern and (2) a striking incidence of lymphocytosis. Most important, however, remains the fact that the subjects in the present study are clinically well and show no evidence of interference with immunological response. These 2 abnormalities could not be correlated with the dosage of any one antigen, number of skin tests received, or occurrence and severity of reaction to the various injections.

The importance of the serum electrophoretic pattern abnormality associated with immunization cannot be readily interpreted. It seems, however, that this protein change could be indicative of an untoward effect of hyperimmunization. The possibility that amyloid deposition might be induced by intense immunization is suggested by the observations in experimental animals, wherein amyloidosis occurs following hyper-immunization (1–19). Over an 8 to 13 year period, however, there is as yet no conclusive evidence that amyloidosis or other organic disease has been induced by repeated inoculation of large doses of antigen in the men studied here.

SUMMARY

The results of clinical and laboratory studies of 99 adult men who were intensively immunized have been presented. Although the group was, on the

whole, well, two findings could probably be attributed to hyper-immunization. These abnormalities include: (1) an abnormal serum electrophoretic pattern and (2) a high incidence of lymphocytosis. Although this is not conclusive evidence of deleterious influence of hyper-immunization in man, the possibility that intensive antigenic stimulation might be associated with untoward effects is considered.

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A SIMPLIFIED EXPLANATION OF THE THEORY OF INDICATOR-DILUTION FOR MEASUREMENT OF FLUID FLOW AND VOLUME AND OTHER DISTRIBUTIVE PHENOMENA[1]

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Principles underlying indicator dilution methods for measuring fluid flow and volume have been considered in a number of reports, reviewed critically and clearly by Dow (1). Nevertheless, because the specialized language of some of the original reports has seemed to obscure their content for many readers, there still appears to be a need for review of these principles in terms that may be more easily understood.

The approach offered is based on the development expressed formally by Meier and me (2) and the exposition employs devices that have proven useful in teaching for the past several years. On the basis of these devices some extensions of the original principles will also be presented.

In general, two techniques have been used to apply indicator dilution principles. In one, indicator is introduced continuously at constant rate into the fluid system; in the other, indicator is introduced suddenly at a single shot. Later it will be shown that other techniques may offer certain advantages in particular settings.

Consider first the case of constant injection of indicator in its simplest form, illustrated by the model in Figure 1. This is a beaker filled with water through which water flows from a reservoir at constant rate. To measure this constant rate of flow of water we add an indicator at a constant rate, I mg./min. An indicator is any quantifiable substance. We ask of it only that it does not change the characteristics of the fluid system and that it remain susceptible to complete quantitative recovery during the period of analysis. Ordinarily the

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Based on and extended from a talk to the Circulation Group during the meetings of the Federation of American Societies for Experimental Biology, April, 1956. Dr. Paul Meier thought of treating the concentration-time curve following injection of indicator as a frequency function of transit times, which is the heart of the analysis. Most of the other ideas in this report stem from discussion with him. Similar treatment was given subsequently and independently by Burger, van der Feer and Douma (5).

indicator is added in solution and it is assumed that the rate of introduction of indicator solution is negligible compared to the flow of the system. If it is not, we simply measure the total flow (fluid plus indicator solution) as described below and subtract from it the known rate of introduction of indicator solution. Returning to the model, we assume that inflowing indicator and water mix thoroughly at some point. The concentration of indicator mixed with water is measured in samples at the outflow from the beaker. The rate at which indicator leaves the beaker is the product of its concentration at outflow, C, and the flow of water, F. After a while all fluid initially present in the beaker is replaced by indicator-laden fluid. The concentration of indicator is then uniform throughout the beaker and equal to its output concentration, C_{max}. When this occurs the overflow of indicator from the beaker exactly equals its rate of inflow, that is, $I = C_{max} \times F$, or flow = rate of constant injection divided by C_{max}, the concentration defined further below.

When the concentration of indicator in the outflow tube is plotted against time, as in Figure 2, concentration builds up to a maximum, C_{max}, and remains there as long as flow is constant. When injection stops, indicator is washed out of the system and concentration falls to zero.

Consider the shaded areas in Figure 2. Reversing the roles of indicator-laden fluid and indicator-free fluid we see that the washout curve is simply the inverse

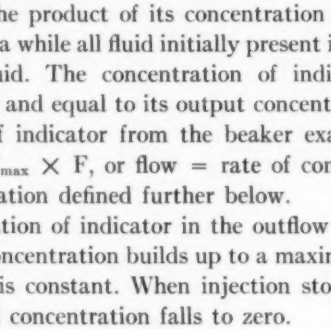

FIG. 1. Simple example of measurement of flow by constant injection of indicator. See text.

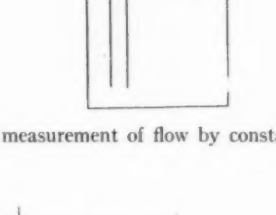

FIG. 2. Concentration of indicator at outflow during and after constant injection. Injection starts at zero time and stops at arrow. Broken line is concentration of indicator at inflow. See text.

of the buildup curve and, therefore, that the two shaded areas are equal. Since the eventual output concentration, C_{max} (dashed line), is equal to the input concentration, C_{max} times the flow is the rate at which indicator is introduced into the system. The solid line represents the concentration at outflow as a function of time and this multiplied by flow is equal to the rate at which indicator leaves the system at time t. Thus flow $\times$ (C_{max} $-$ C) is the rate at which the amount of indicator in the system increases at time t. The area above the build-up curve multiplied by flow is the total quantity, Q, of indicator contained in the system at the time equilibrium is established, that is, at the time the rate of entrance and rate of exit of indicator are equal. This quantity of indicator is distributed uniformly at concentration, C_{max}, in some volume, V. Since concentration is mass divided by volume, the volume of the system, V = (area above the build-up curve) $\times$ F/C_{max}. Later it will be shown that V = (mean transit time) $\times$ F. If this is true then it will have to be shown that (area)/C_{max} is also exactly the mean transit time, a term to be defined.

In order to do this we will consider first the principles underlying the use of a single sudden injection of indicator. We will then show that the single-injection and the constant-injection concentration-time curves are equivalent in the sense that one can be derived from the other and the temporal events of physiologic interest in the single-injection concentration-time curve are simultaneous with those of the constant-injection curve. This identity was first described accurately by Hamilton and Remington (3). Further proof of the identity was supplied by Meier and me by starting with the proposition that the single-injection indicator-dilution curve is simply a frequency function of transit times that make up blood flow.

A. INDICATOR CONCENTRATION AS A FREQUENCY FUNCTION OF TRANSIT TIMES

Frequency functions may be described by the following example. Suppose we wish to know during how many days a year snow fell in Baltimore. On reviewing snowfall records for the past 12 years we find there were in each year 3, 3, 4, 4, 4, 4, 5, 5, 5, 6, 6, and 7 days of snow, not necessarily in that order. To find the mean number of days of snowfall per year we can simply add up the 12 numbers and divide by the number of years and the mean is 56/12, or about 4.7 days.

Alternatively we can display the data as shown in Figure 3. The abscissa is the observed number of days of snowfall in any year. The ordinate shows how often the same number of days of snowfall occurred among the sample of 12 years, that is, it is the frequency with which an event occurred. In the example there are 12 years. The width of each square, Δt, is unity, the height of each square is assigned the number 1/12, the area of each square is therefore 1/12 and the sum of the areas of all squares is 12/12 or unity, as it must always be

since the total area represents all the observations. The area of each column, or frequency times Δt, is multiplied by time, or the number of days of snowfall, and these products are summed. The answer is the mean, 56/12. The mean, then, is defined as the sum of all products of frequency multiplied by Δt multiplied by time.

In Figure 4 is shown the same arrangement of squares as in Figure 3 but the

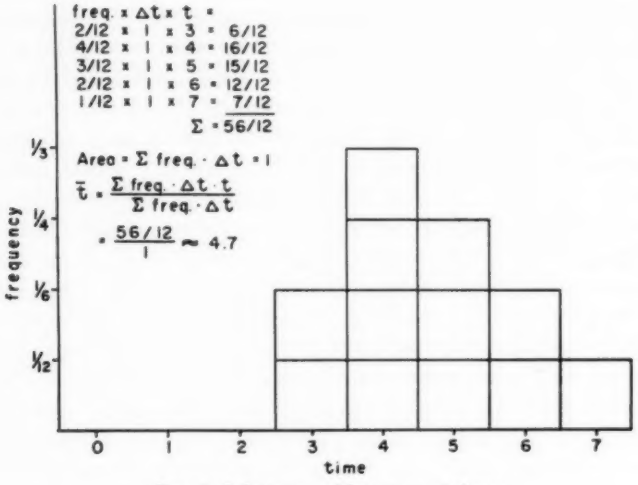

FIG. 3. A frequency histogram of times.

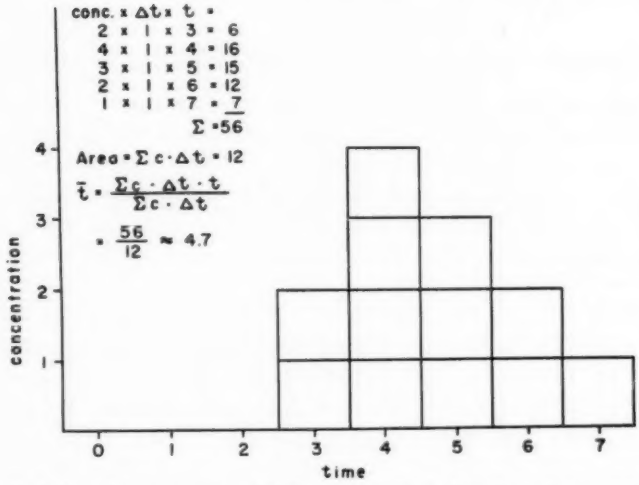
conc. x Δt x t =
2 x 1 x 3 = 6
4 x 1 x 4 = 16
3 x 1 x 5 = 15
2 x 1 x 6 = 12
1 x 1 x 7 = 7
Σ = 56
Area = Σ c · Δt = 12
t̄ = Σ c · Δt · t / Σ c · Δt
= 56/12 ≈ 4.7
concentration
4
3
2
1
0 1 2 3 4 5 6 7
time

FIG. 4. Variation of concentration of indicator with time plotted as a frequency histogram. Distribution of transit times same as in Fig. 3.

ordinate has been multiplied by a constant. This is now the concentration-time curve resulting from single injection of indicator. At zero time a quantity of indicator is introduced into a vascular bed. After a while some indicator appears at the sampling site. If the indicator was well-mixed with total flow through the bed, then at any moment the total flow carries out of the bed an amount of indicator described by flow times concentration. Eventually all the injected indicator leaves the bed; that is, the sum of the columns multiplied by flow must equal the quantity of indicator injected, or flow can be measured as the known quantity of indicator injected divided by the area of the single-injection concentration-time curve.

The fraction of injected indicator leaving the bed at any time is the quantity carried away at that time, or concentration multiplied by flow, divided by the quantity introduced. Therefore, to convert the observed concentration-time curve to a frequency distribution curve multiply the units on the ordinate scale by a constant, flow divided by quantity of indicator injected.

To calculate the mean transit time from the concentration-time curve, multiply each concentration by the time interval and by its transit time, sum these products, 56, and divide by the area of the curve, 12, and the answer, as before, is 56/12. If each concentration were divided by the area of the curve and this number were multiplied by the time interval and by the transit time, the sum of these products is also the mean transit time because concentration at any time divided by the area of the curve is exactly the frequency.

B. MEASUREMENT OF VOLUME FROM FLOW AND MEAN TRANSIT TIME

The volume of the vascular bed can also be calculated from the single-injection concentration-time curve. First consider that a vascular bed, whether a single chamber, a single vessel, or a network of many vessels, is described by a family of transit times, that is, by the various times required for blood to traverse it. In Figure 5 blood is represented as entering the bed from the left to be distributed over a number of different paths which finally merge to a common outflow. Some blood particles move from entrance to exit in a shorter time than do others, either because their path length is shorter or their linear velocity is greater, or both. The numbers in Figure 5 indicate the transit time in seconds for a particle to move from entrance to exit along the path indicated and the numbers have been chosen to give the frequency distribution of

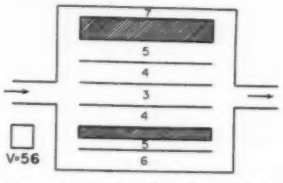

FIG. 5. Vascular bed as a family of transit times. See text.

transit times used in Figures 3 and 4. At the moment the experiment is begun the system is already filled with blood and we wish to know its volume. The little square in the lower left hand corner of Figure 5 is the unit volume and there are 56 squares in the system.

Indicator is injected at zero time in such a way as to tag all inflowing blood completely and uniformly. Tagged particles will have the same transit times as untagged particles already in the system.

Figures 6 to 9 are a series of photographs of a three-dimensional model made of cubes. Black cubes represent indicator-tagged blood; white cubes are untagged blood. The axis coming toward the reader is experimental elapsed time. After injection of indicator at zero time, none appears during the first two seconds. During the third second 2/12 of the injected tag appears. This fact is shown by the height of the column on the Y-axis, which is multiplied by the frequency function, h(t); that is 2/12 of the total flow has a transit time of three seconds. During the fourth second, 4/12 of the injected particles appear. And so on, until all the injectate is accounted for. Figure 6 illustrates that the experimental elapsed time at which indicator particles appear is the same as the transit time for these particles and this fact is indicated by the ordinate scale running from left to right. The usefulness of the indicator lies exclusively in the fact that the distribution of transit times for indicator particles can be measured from the experimental elapsed time and is assumed to be the same as the distribution of transit times for all blood, or, more strictly, of all untagged particles of the same type as tagged particles.

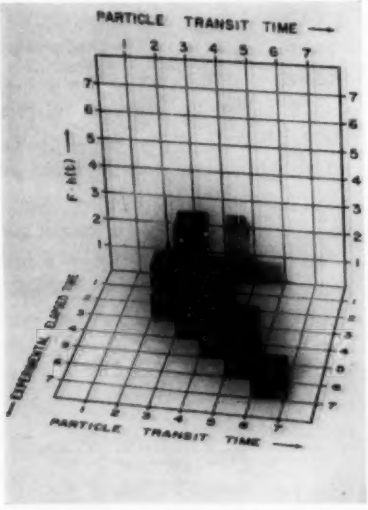

FIG. 6. Indicator particles as markers of transit time. Black cubes represent indicator particles. Distribution of transit times same as in Fig. 5.

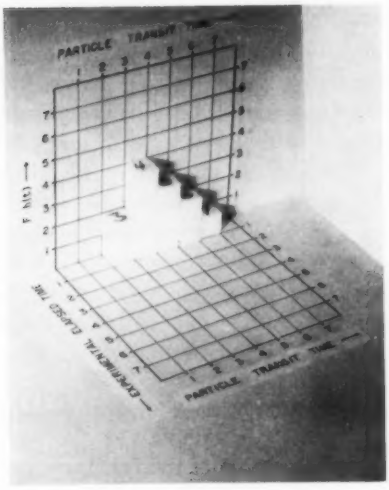

FIG. 7. Total flow during first time unit following injection of indicator. White cubes are untagged particles. Numbers on the top of each column of cubes indicate transit time of the cubes in that column.

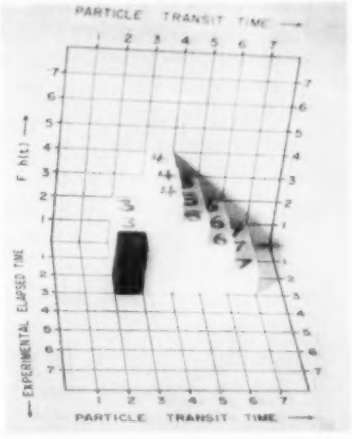

FIG. 8. Total flow during first three time units following injection of indicator. Fastest indicator particles (black cubes) have just appeared.

Figure 7 illustrates the force of this fact. The 12 cubes untagged by indicator represent the total outflow during the first second following injection of indicator. It is known from the experimental indicator-dilution curve that 2/12 of the outflow during any interval of time is made of particles that entered the vascular system three seconds earlier, 4/12 entered four seconds earlier, 3/12 entered five seconds earlier, and so on.

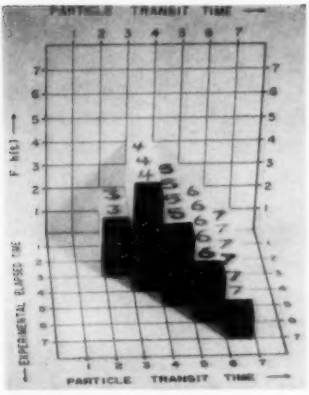

FIG. 9. Measurement of volume by single injection of indicator. See text.

In Figure 8 are displayed the events of the first three experimental seconds. During the third second the first indicator particles, shown as black cubes, appear. They constitute 2/12 of the total flow during that second; the remaining 10/12 of the flow is made of undyed particles. Look down the row of cubes whose transit time is between two and three seconds. It contains 2×3 or 6 cubes. This means that at zero time, when indicator was introduced into the system, that part of the vascular volume composed of particles described by 3-second transit occupied a volume of 6 units. It is as though indicator particles have flushed out ahead of them all untagged particles of the same transit time which were already in the vascular bed at zero time.

Figure 9 shows the events of the first seven experimental seconds, at the end of which all injected indicator has been washed out of the vascular bed. As one looks down the rows of cubes in each interval of transit time, one sees that indicator particles at exit have been preceded by the fraction of the volume of the vascular bed occupied by untagged particles having the same transit time. The sum of all such fractional volumes is the total volume of the bed.

The volume occupied by each row of cubes is the product of flow and the distribution function (a partial product that describes the fraction of the total flow having a given transit time) and the transit time and the time interval. The sum of all rows is the total volume of the vascular bed; it is the sum of all products of F, h(t), t and Δt, and this will be recognized from the discussion of Figure 3 as exactly the product of flow and the mean transit time. This, then, is a proof that volume is flow multiplied by mean transit time.

Figure 9 also helps make clear the assumptions underlying indicator dilution methods. It is assumed that (1) the distribution of transit times for indicator particles is the same as that for all blood (or for all particles of the type for which flow and volume are to be measured) flowing through the system,

(2) total flow through the system does not change during the experimental period between injection of indicator and recovery of all indicator, (3) total volume of the system does not change during the experimental period, and (4) there is stationarity; that is, the distribution of transit times does not change during the experimental period. Finally, it is assumed that recirculation either does not occur or that it can be corrected for experimentally.

It is now possible to consider the case of continuous injection of indicator at a constant rate from the point of view of frequency distribution of transit times. The relation between outflow concentrations of indicator associated with single and constant injection can be seen easily if one imagines that, in Figure 9, once indicator-tagged particles appear at any given transit time, they will continue to appear with the same frequency at that transit time so long as injection continues.

The cubes may be re-arranged, as in Figure 10 to illustrate the shape of the outflow concentration-time curve during constant injection of indicator. Figure 10 shows 12 cubes representing the total outflow from the system. We know from the single-injection curve, Figure 6, how the transit times making up total flow are distributed: 2/12 of the flow travels the bed in three seconds; an additional 4/12 travels in four seconds, or 6/12 travels in four seconds or less; an additional 3/12 travels in five seconds, or 9/12 travels in five seconds or less; and so on until the full 12/12 of the flow is accounted for. The ordinate, then, is the cumulative frequency function, H(t), multiplied by flow.

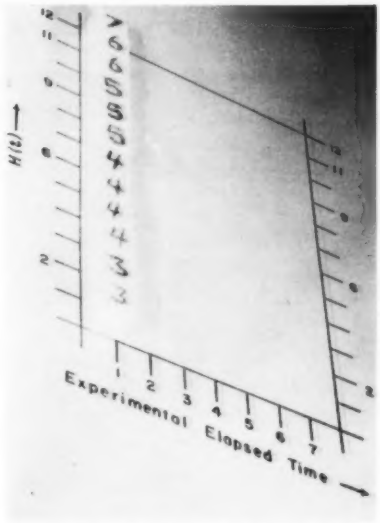

FIG. 10. Cumulative frequency function. Same distribution of transit times as in previous figures. White cubes are untagged fluid particles.

Figure 11 illustrates the time course of appearance of indicator particles during constant injection, begun at zero time. No indicator appears until the third second when 2/12 of the flow—the fastest particles—is tagged and will remain tagged as long as constant injection continues. During the fourth second there appear tagged particles of the kind that require four seconds to traverse the bed, so that a total of 6/12 of the flow is tagged. And so the constant injection curve is developed until all the outflow is tagged, at which plateau we have a measure of total flow. Note that it takes exactly as long for the concentration-time curve during constant injection to reach plateau as it takes that following single injection to fall to zero.

The total number of cubes in Figure 11 is 56. They are the same 56 cubes that made up the total volume in Figure 9, which illustrated events following single injection of indicator. Note that in Figure 11 the frequency of 3-second particles multiplied by their transit time gives six 3-second particles; the frequency of 4-second particles multiplied by their transit time gives 14 4-second particles. Thus, the area above the build-up of the constant-injection curve is the sum of the products of frequency, time and Δt, which is the definition of the mean transit time. Earlier in this paper (see Fig. 2) it was shown that volume equals flow multiplied by the area above the build-up curve. Since it has now been shown that this area is exactly the mean transit time we have independent proof of the fact that volume is flow multiplied by mean transit time.

In the models used here the vascular bed was marked by only four types of transit time, from three to seven seconds. In real vascular beds there is a very large number of types of transit time so that a plot of their frequency

FIG. 11. Measurement of volume by constant injection of indicator. Black cubes are tagged particles. See text.

distribution yields a more or less smooth curve. Following sudden single injection of indicator into a vascular bed, the concentration at outflow usually increases rapidly, after a brief initial upward concavity, to a maximum and then declines more slowly and curvilinearly to zero. Peak concentration usually occurs at some time less than half the total time required to write the curve, that is, the curve is skewed to the left toward shorter transit time and the mean transit time is located on the down-limb. It was pointed out by Hamilton, Moore, Kinsman and Spurling (4) that the down-limb often seems to fit an expression for exponential decay, that is, the rate at which the concentration falls at any time is proportional to the concentration at that time. While this has undoubtedly been a useful approximation the fact is that the early portion of the down-limb falls more rapidly than its middle portion which does very often fit the expression for exponential decay rather well, and the later portions of the down-limb are so frequently obscure experimentally that it is not clear whether or not they conform. It will be shown later that the practical applications of Hamilton's approximation are so simple and valuable that the fact that the down-limb is not an exponential form does not matter for many measurements of flow and volume.

C. RELATION BETWEEN SINGLE AND CONSTANT INJECTION OF INDICATOR

There have been a number of ingenious efforts to supply formal expression on *a priori* grounds for the single-injection indicator dilution time curve, reviewed by Dow (1), but these appear to be doomed to failure in the present state of our ignorance about the characteristics of the bed. It has seemed best for the time being to let the bed describe itself to us by writing its own frequency distribution curve. The frequency of transit times is related to the concentration of indicator following single injection by the fact that the fraction of indicator leaving the bed at any time is described exactly both by the product of flow, F, and concentration, c(t), and by the product of frequency at which a given transit time occurs, h(t), and the quantity of indicator injected, or $q \cdot h(t) = F \cdot c(t)$. For continuous injection at constant rate the cumulative frequency function at a given time, H(t), is to the total flow as the concentration at that time, C(t), is to the rate of constant injection, I; that is, $H(t)/F = C(t)/I$. Since H(t) grows only until it is unity, at which time C(t) becomes the plateau concentration, C_{max}, this expression confirms the intuitive formula given earlier, $F = I/C_{max}$. Furthermore, from the relations concerning the single injection curve, the complete area under the curve of frequency distribution of transit times, conveniently written $\int_0^\infty h(t)\,dt$, multiplied by q represents the total quantity of indicator injected (since the total area under the frequency curve is unity) and this equals $F\int_0^\infty c(t)\,dt$, which is the expression for determining flow given in discussion of Figure 4.

Figure 12 illustrates simultaneously obtained curves resulting from single

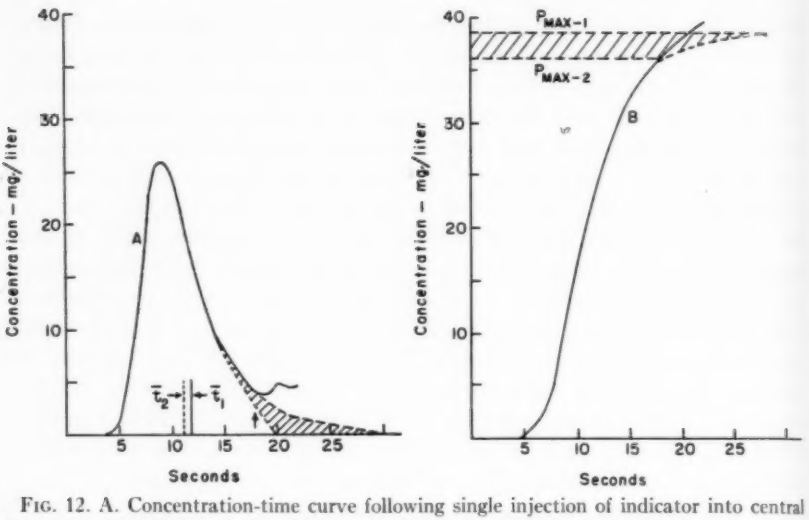

FIG. 12. A. Concentration-time curve following single injection of indicator into central circulation (solid lines). Broken lines represent likely extremes of extrapolation of concentration to zero during the first circulation of indicator. Recirculating indicator just appears at the arrow. Shaded area is relative difference between two estimates of flow based on the two extrapolations. $\bar{t}_1$ and $\bar{t}_2$ are the two estimates of mean transit time based on the two extrapolations.

B. Concentration-time curve following constant injection of indicator into central circulation. Time at which indicator just appears at outflow and time at which indicator just begins to recirculate are identical to those shown in curve A. P_{max-1} and P_{max-2} are likely alternative extremes of extrapolation of concentration to plateau. Shaded area is difference between estimates of area above the extrapolated build-up concentration curves.

injection, curve A, and constant injection, curve B, into the central circulation, including the heart and the pulmonary vascular bed. The quantity of indicator has been adjusted to yield reasonable peak and plateau concentrations. All temporal events of interest occur simultaneously in the two. The times at which indicator first appears are the same. The initial phase of the constant injection curve is concave upward followed by a phase concave downward until plateau is reached. The point at which concavity changes from upward to downward coincides with the time at which the single-injection curve reaches its peak. Recirculation of indicator, represented by departures from smooth curves, occurs simultaneously. The dashed lines represent extrapolation to zero concentration for curve A and to a plateau concentration for curve B; that is, they are corrections for recirculation and show that curve A returns to zero at the instant curve B reaches plateau. It is as easy to make one extrapolation as the other. If recirculation is a late event, as shown in Figure 12, so that curve A has almost returned to zero, then no reasonable, even if crude, extrapolation is

likely to lead to gross error since most of the area has already been written. Similarly, if curve B is very near plateau it would be difficult to be wrong about the extrapolation. On the other hand, if recirculation occurs so early that no reasonable idea of the shape of the down-limb of the single injection curve is obtained, neither curve can be extrapolated with confidence.

Volume is apt to be estimated less precisely than flow. For example, if the extrapolation to zero of curve A is falsely early, leading, say, to a 10 per cent underestimate of area (and therefore to a 10 per cent overestimate of flow), mean transit time ordinarily will be underestimated more seriously, say by 20 per cent, and volume, which is the product of overestimated flow and more greatly underestimated mean transit time, will be underestimated, in this case by 12 per cent.

Similarly, in curve B, if plateau concentration is underestimated (and flow overestimated) the area above the curve, required for calculation of volume, will be more seriously underestimated, and volume will therefore be underestimated, and, in general, to a greater degree than the overestimate of flow.

D. ALTERNATIVE FORMS OF INJECTION, NEITHER SINGLE NOR CONSTANT

Let us now consider effects of variation in rate of injection. So long as the system is stationery, that is, flow, volume and frequency distribution of transit times are constant, the concentration-time curves following single injection of each of two quantities of indicator will stand exactly in relation to one another as the relative quantities of indicator injected. For example the injection of twice the quantity of indicator produces twice the concentration at any time, and twice the area from time zero to any given time with no change in the time course. This trivial statement follows immediately from the fact, demonstrated above, that $c(t) = q \cdot h(t)/F$, for if we now inject twice the quantity, the new concentration function $c_1(t) = 2q \cdot h(t)/F$.

If two different quantities of indicator are injected in rapid succession so that the concentration change produced by the second injection appears before the first indicator has been washed out of the system, the concentration measured will be the sum of the two concentrations each injection would have produced separately. If, instead of two discrete injections, we inject continuously at a rate which is a function of time the concentration of indicator accumulates in the general manner of the concentration associated with constant injection, but modified by the fact that the amount of indicator injected over any interval of time may be different from time to time.

An interesting and possibly useful result occurs if injection is continuous at a rate which increases linearly with time. The principle is illustrated in Figure 13. During the first time interval an amount, say q_1, of indicator is injected and is distributed in a series of concentration each equal to $q\ h(t)/F$, as shown by the

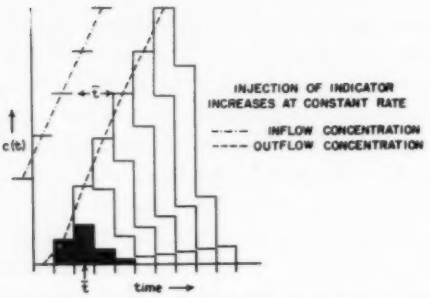

FIG. 13. Measurement of mean transit time by constant acceleration of injection of indicator. See text.

black rectangles. During the next time interval an amount, say $a \cdot q_1$, where a is greater than 1, is injected and is distributed in a series of concentrations each equal to $a \cdot q\ h(t-1)/F$, as shown by the white rectangles added to the first black ones where $h(t-1)$ is zero for t less than 1. In the third time interval an amount $2 \cdot a \cdot q$ is injected and distributed in accordance with the frequency function, $h(t-2)$, and so on. We do not in fact measure the separate contribution of each of these quantal injections; we measure only their cumulative effect as the concentration at any moment. Notice that the increment in measured concentration with succeeding time intervals is irregular for a period of time following which each successive concentration exceeds its predecessor by a constant amount. The time at which constant increments begin is exactly the time at which h(t) just returns to zero, or, in Figure 13, the time at which the quantity of indicator injected during the first time interval is just washed out of the system. Recall that if we had been injecting at constant rate this is also the time at which the concentration would have reached plateau.

Let us generalize the evolution of the curve in Figure 13. At any time, t, the concentration is made of a number of groups of indicator particles which entered the system at different times before t. If the rate of injection of indicator is i(t), the quantity injected during the interval, ds, beginning s time units before t is $i(t-s) \cdot ds$, and the fraction of it leaving at t is h(s). Then the rate at which indicator particles of this kind leave the system at time t is $i(t-s) \cdot h(s) \cdot ds$, and their contribution to the concentration is this quantity divided by total flow. The total concentration at time t is the sum of all such particles where s varies from zero to t. That is, as pointed out previously (2),

$$C(t) = \frac{1}{F}\int_0^t i(t-s) \cdot h(s) \cdot ds.$$

If, as in Figure 13, i(t) is linear with time, that is, $i(t) = t \cdot a(t)$, where $a(t) = 0$ for $t < 0$ and $a(t) = a$, a constant, for $t \geq 0$, then, substituting $(t-s)a$ for $i(t-s)$ in the equation for concentration, gives $C(t) =$

$\frac{at}{F}\int_0^t h(s)\,ds - \frac{a}{F}\int_0^t s \cdot h(s) \cdot ds$. As t grows large the first integral approaches unity, since the frequency distribution curve has unit area, and as t grows large the second integral, it will be recognized, approaches the mean transit time. That is, with large t, $C(t) = (t - \bar{t})a/F$, where $t > \bar{t}$. Furthermore, we know exactly how large t must be when this linear expression for C(t) just becomes valid; it must be that time at which $\int_0^t h(s)\,ds = 1$, that is, the time at which the concentration following single injection just returns to zero.

When one injects at rate i(t), the concentration at the point of injection, if indicator is mixed with total flow is i(t)/F. If the rate of injection is linear, say t a, concentration at entrance is t a/F. Comparing this value for inflow concentration with that given above for outflow concentration, one sees that the concentration of outflow, for sufficiently large t, is the same as the concentration was at inflow $\bar{t}$ time units earlier. Thus, once the concentration at outflow begins to increase linearly, the difference between it and i(t)/F is $\bar{t}$, a simple method for measurement of $\bar{t}$.

There are two important examples of the effect of continuous, not necessarily constant, introduction of indicator, i(t), upon the concentration of indicator at outflow, c(t).

One example concerns the question of how instantaneous an instantaneous injection must be. If an injection, intended to be "instantaneous", is in fact distributed over some period of time as illustrated by the frequency distribution on the left in Figure 14A, then the mean time determined by analysis of c(t) will exceed the mean time of untagged particles, which should be that of the

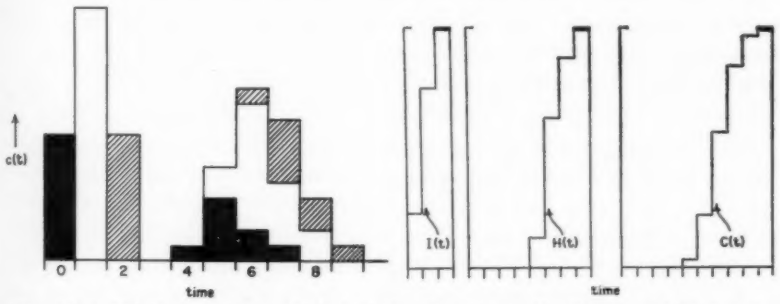

FIG. 14. A. Non-instantaneous single injection. Frequency distribution of injection shown between 0 and 2 time units. Frequency distribution of transit times shown by black columns between 4 and 6 time units. The same frequency distribution resulting from each quantum of injectate is superimposed on its predecessor. The envelope is the concentration at outflow measured in fact.

B. Cumulative frequency function of injection, I(t), distribution of transit times, H(t), and concentration at outflow C(t) from injection shown in Fig. 14A.

distribution function h(t), shown as solid blocks on the right in Figure 14A. In fact the mean time of the function i(t) plus the mean time of the function h(t) equals the mean time of the function c(t). This relation is illustrated in Figure 14B which presents cumulative frequency distributions of i(t), h(t) and c(t), in that order. Recall that the area above the cumulative frequency distribution function is the mean time and note that the sum of the areas above I(t) and H(t) equals the area above C(t), where capital letters denote respective cumulative functions. Therefore, as pointed out in a previous report (2), the mean transit time of untagged particles of the kind whose flow and volume are of physiological interest is obtained by subtracting the mean time at which indicator is injected from the mean time estimated from concentration of indicator at outflow. If the mean time of injection is relatively long compared to mean transit time, accuracy of estimate of the latter may be compromised.

E. RECIRCULATION AND SOME APPLICATIONS OF INDICATOR-DILUTION THEORY TO OTHER PROBLEMS

The second example concerns recirculation. In a closed system, that is, one in which iterated recirculation occurs, following single injection of indicator, the system reinjects itself continuously. The shape of the continuous reinjection is that of the frequency function of transit times through the whole circulation system.

The simplest case, illustrated in Figure 15A, is that in which the system under study is the whole circulation; that is, arbitrary outflow at which concentration of indicator is measured is immediately proximal to arbitrary inflow at which indicator was introduced and indicator, once introduced, is not removed from the system. There is, therefore, only one distribution function of transit times, h(t), and the quantity of indicator at outflow at any time t is formed by summing the elements $h(t - s) \cdot h(s) \cdot ds$, as shown in the discussion of Figure 13. As s moves from time 0 to t, h(s) includes low and high frequencies so that wide swings in concentration of indicator at outflow tend to decrease; that is, the concentration function behaves like a damped oscillation (Fig. 15B). With time, indicator leaving the outflow reenters the inflow at a rate which is progressively more like a constant injection and it is intuitively clear that eventually indicator will in effect be distributed uniformly throughout the system. The concentration at which this occurs is the quantity of indicator introduced divided by the volume of the system. Indeed this is one well-known method for determining volume of distribution of indicator at a steady concentration.

If the system under study is only part of the circulation, and if h(t) is the frequency distribution of transit times through the partial circulation and f(t) is that through the whole circulation, then it is obvious from the discussion

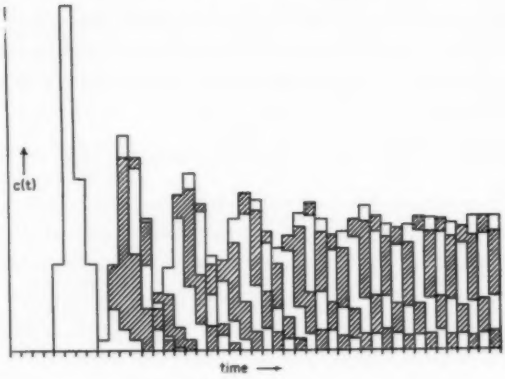

FIG. 15. A

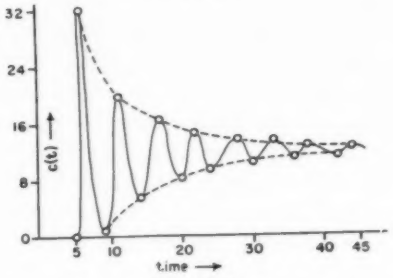

FIG. 15. B

FIG. 15. A. Recirculation of indicator following single instantaneous injection. Envelope is measured concentration of indicator. First four columns represent initial circulation. As indicator reinjects itself into the system each slug is distributed according to the frequency pattern illustrated during the first circulation. Distribution of successive reinjected slugs is marked by alternate white and shaded patterns. For example, the second slug to reinject itself happens to be that with the most frequent transit time and its distribution on recirculation is shown by the first large shaded pattern.

B. Recirculation as a damped oscillation. Envelope of Fig. 15A is shown by the solid line. The beat of this oscillation is shown by the broken lines joining maxima and minima respectively.

presented so far that $c(t) = (q/F)\int_0^t f(t - s)h(s)\,ds$. Since the mean time of the distribution f(t) adds to the mean time of the distribution h(t) to yield the mean time of the distribution estimated from c(t), it is easy to determine the third of these mean times if the other two can be measured.

If at some time, say T, C(T) becomes constant, as suggested by Figure 15, $C(T) = (q/F)\int_0^T f(T - s)h(s)\,ds = q/V$, Since $V = F\bar{t}$, $\int_0^T f(T - s)h(s)\,ds = 1/\bar{t}$.

It is difficult to obtain f(t) or h(t) if only one of these and C(t) are known, but if two of the three functions are well-described it should be possible to obtain the third function by trial and error or by computer methods from the relations described above.

The relation $c(t) = \frac{1}{F}\int_0^t i(t - s)\cdot h(s)\, ds$ probably has wide application in biology since it is simply the convolution of one distribution upon another. In neurophysiology it describes the distribution of arrival times of nerve impulses, for example, that have traveled through a family of synapses and then been conducted along a family of axones. In metabolic studies it describes the concentration of a metabolite produced by a family of cells and distributed over some volume in the organism or distributed over some volume in the organism and dissimilated by family of cells. If one of the distribution functions can be measured independently, measurement of the function c(t) provides a possible method for estimating the remaining function.

It is interesting to explore the effects of violation of the basic assumptions of indicator-dilution methods. If, for example, flow is not constant but is a function of time, F(t), then the distribution of transit times will be a function of flow, h(F, t). It must still be true that, following a single injection, the quantity of indicator leaving the system at any time is $F(t)\cdot c(t) = q\cdot h(F, t)$. It is therefore impossible to measure flow by this method alone. However, if flow varies in some systematic way and sufficiently slowly so that h(F, t) can be estimated satisfactorily, say be repeated injection of indicator at intervals of time F(t) may be estimated with some knowledge of the error involved. Some notion of possible relations can be gotten by constructing frequency distribution graphs and letting the distribution vary with time or by manipulation of the model made of cubes used in earlier illustrations. If flow is measured by an independent method, then h(F, t) obviously can be determined easily. Volume, however, cannot be measured because indicator particles under these conditions do not necessarily flush out untagged particles of the same transit time.

The assumption of stationarity may be untrue. Microscopic examination of living vascular beds, for example that in the frog's tongue, shows what appears to be harried reshuffling of the distribution of blood flow among the several channels. Under these circumstances even though flow is constant the distribution function varies. If knowledge of the distribution function is desired it may be possible to investigate its behavior by repeated single injection of indicator and independent measurement of flow.

Further discussion of the effect of violation of the assumptions underlying indicator dilution methods has been presented previously (2).

Finally, by proper selection of injection and sampling sites and of indicator, it may be possible to learn a good deal about the system under study. Thorough experimental description of the distribution of transit times may lead to some

understanding of the character of flow through the system, the distribution of path lengths, detection of shunts, of barriers to flow or to diffusion. Proper combinations of indicators, using the word broadly, may provide descriptions of the separate distributions making up a single system and the technique can be, and already has been, extended to examine other biological phenomena besides fluid flow and volume.

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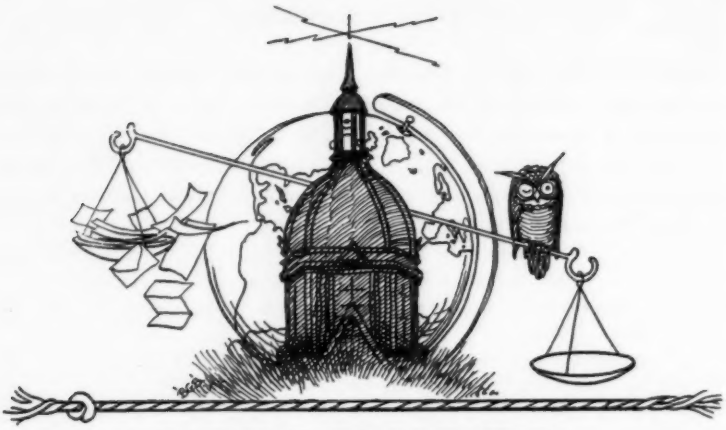

BOOK REVIEWS

Obstetrics and Gynecology. By J. Robert Willson, Clayton T. Beecham, Isador Forman and Elsie Reid Carrington. 605 pp., $10.75. The C. V. Mosby Co., St. Louis, Mo.

This book on obstetrics and gynecology affords ample proof of what every thinking individual should already be aware—the specialties of gynecology and obstetrics have simply become too complex and replete with too many ramifications to allow for adequate specialty coverage whether it be within a combined department or in a combined textbook. This does not, however, detract from its general excellence or the stated intent by the authors to produce a publication that can be utilized by the family physician. In this purpose they have succeeded admirably, but there is no question, as the authors admit, that the specialist will not be satisfied with this, or with any single textbook.

In the 51 chapters and 590 pages these four Temple University authors have covered all of the important aspects of both specialties, of necessity in a rather condensed form. For example, the very interesting and provocative problem of pelvic tuberculosis is handled in only two pages. The first eight chapters are devoted primarily to normal physiology and such functional problems as amenorrhea, dysmenorrhea, sexual problems in the female, etc., and these have been covered quite adequately. Rather disappointing was the chapter on endometriosis. This lacked an up-to-date bibliography, and unlike many of us, Dr. Beecham does not seem to place much credence in the factor of tubal regurgitation as a factor in the development of many cases of endometriosis. His other contributions, particulary on hydatidiform mole and choriocarcinoma are excellent.

The middle third of the book is devoted exclusively to obstetrics, and while this reviewer admits to being only a gynecologist, helpful obstetricians have assured me that it has been done in the high calibre characteristic of all of Dr. Willson's publications. The final chapters deal with organic pathology found generally in the older age group, and although all of the usual disease entities are included, space limitations have necessitated a rather cursory discussion of some of these. The authors have wisely omitted any description of surgical technique, although anesthesia and operative care are covered.

Rather unique and enjoyable is the sequential presentation of the life history of the usual woman with her pregnancies and diseases. Normal physiology, functional disturbances, infections, pregnancy with post-partum complications, tumors, etc., are logically presented in their usual order of appearance in a woman's life.

The photographs, many of which are second hand and diagrammatic or semi-diagram-

matic, are only average, and hardly in keeping with the excellent literary style. Presented in pleasant format by the C. V. Mosby Company, this is an easily readable book. Any critical comments made in this review are intended only for the more discriminating specialist. For the student and general practitioner who performs office gynecology and obstetrics in the non-teaching type of institution, this text is ideally suited.

EDMUND R. NOVAK

Applied Physiology of the Eye. By H. WILLOUGHBY LYLE assisted by T. KEITH LYLE. 341 pp., $9.00. Bailliere, Tindall and Cox, London, England. The Williams & Wilkins Co., Baltimore, Md. exclusive U. S. agents.

It is indeed fortunate that Doctor T. Keith Lyle saw to it that his father's manuscript was prepared for publication. The book will continue to be a valuable monument to the author's industry and extensive knowledge.

In the preface the objective was stated: To present the main facts concerning the physiology of the eye and to coordinate these with disease states and abnormal functionings. The objective has been obtained in by far the largest part. There are some omissions which this reviewer regrets. For example, fundamental work by Hering, by Head, by Cogan and by Cannon well might have been mentioned. However, this criticism is minor because the mass of material which is presented is sound and is well arranged.

All ophthalmologists and particularly those who are undertaking Board examinations should have this book.

The Williams & Wilkins Co., Baltimore, Maryland, are exclusive U. S. agents.

FRANK B. WALSH

Rehabilitation of the Cardiovascular Patient. By PAUL DUDLEY WHITE, HOWARD A. RUSK, PHILIP R. LEE AND BRYAN WILLIAMS. 176 pp., $7.00. McGraw-Hill Book Co., New York, N. Y.

Rehabilitation of the Cardiovascular Patient presents a concise and relatively comprehensive discussion of all phases of rehabilitation in patients with cardiovascular diseases.

The emphasis is on the physical medicine aspects of rehabilitation, but the emotional and psychological aspects of each disease, its impact upon the patient, and in many cases his family as well, are considered.

Photographic illustrations showing exercises, especially for the hemiplegic patient, are very clear and should be useful to those physicians treating patients who have sustained cerebrovascular accidents.

Optimistic and encouraging statistics on rehabilitation are given, in patients who had sustained myocardial infarctions. Eighty-four per cent of five hundred patients in one study, and 79 per cent of one hundred and eighty-four patients in another study were able to return to work after an appropriate interval and application of rehabilitation principles.

Even more encouraging are the findings of Greer at the Gillette Safety Razor Company in Boston, which indicate that when patients with coronary heart disease are properly placed, they are not only productive employees, but actually rate a little higher in productivity than the healthy controls. The authors feel the same findings obtain in patients with other forms of heart disease who are properly evaluated and placed according to degree of disability, age, sex, economic status, and motivation.

This is certainly a useful contribution by four well known investigators in the field of rehabilitation. All physicians who treat patients with cardiovascular disease occasionally or constantly should find it extremely interesting and clinically useful.

JULIAN W. REED

The Brain and Human Behavior. Research Publications of the Association for Research in Nervous and Mental Disease, Vol. 36. Edited by HARRY C. SOLOMON, STANLEY COBB AND WILDER PENFIELD. 564 pp., $15.00. The Williams & Wilkins Co., Baltimore, Md.

This volume of the transactions of the 1956 meeting of the Association for Research in Nervous and Mental Disease, in general, analyzes the contributions of the various parts of the brain to behavior rather than the characteristics of the total integration such as might have been derived from philosophic and psychodynamic considerations. Thus the research done on frontal, parietal, and temporal lobes in the past decade is assembled and an attempt made to show how it elucidates behavioral mechanisms. A few studies on neuropharmacologic subjects are included.

The detailed studies from the Boston Psychopathic Hospital on lobotomized patients are briefly summarized by Greenblatt and Solomon who discuss their current thinking of the rationale of lobotomy without formulating a generally acceptable thesis.

The influence of the parietal lobe on behavior is well outlined, both from the clinical and experimental standpoints, in an 80 page paper by Drs. Denny-Brown, and Chambers. This must be read to appreciate the detailed wealth of material.

The greater part of the remainder of the book deals with the temporal lobe—the effects of its stimulation and ablation upon behavior. The concepts presented largely fall in line with Penfield's thesis of a temporocentrencephalic interrelationship.

This book presents the current thinking of brain function by the experimental and clinical neurophysiologist. By no means does it give an acceptable or total analysis of behavior. Perhaps its general thesis might be epitomized by the conclusions of the authors of the last paper in the monograph—Dr. Chapman and associates—who state that the site of cerebral damage is important in respect to low (motility and sensation) and intermediate (speech etc.) level functions but the form and degree of impairment of the highest integrative functions is independent of the site of tissue loss within the neopallium but closely related to the mass of improperly functioning neural tissue. Moreover the degree of impairment is greater in patients with pre-existing anxieties and difficulties in over-all adaptation, than in those who performed exceptionally well. In fact, neuronally intact individuals with long standing and severe anxiety, manifested by difficulties in mentation, easy frustration and fatigability have impairment of the higher mental functions, sometimes, to the same degree as those patients with major loss of cerebral tissue.

This volume should be read by all serious students of neurology for it presents concisely current concepts of mind-brain relationship.

A. EARL WALKER

Physiology of Prematurity, Transactions of the First Conference, March 21, 22 and 23, 1956. Edited by JONATHAN T. LANMAN. 151 pp., $3.25. The Josiah Macy, Jr. Foundation, New York, N. Y.

I believe that this 135-page textbook will appeal more to the academic obstetrician and pediatrician than to those engaged in practice—an unfortunate circumstance, for all of us can profit by reading the proceedings of this first conference on prematurity. The two subjects presented are Fetal-Maternal Endocrinology in Late Pregnancy and The Fetal and Placental Circulation in Late Pregnancy. The discussions are opened very ably by Drs. Jonathan T. Lanman and Geoffrey S. Dawes. The comments of the participants are of much value and in many ways make this little text a most enjoyable one to read. Another attractive feature is the current bibliography which appears at the end of each chapter.

Today one hears a great deal about the value of the multidisciplinary approach to problems in obstetrics. As working proof of this idea, one has only to analyze the group attending

this meeting. Of thirty participants, only three were obstetricians. I recommend this book to all those interested in prematurity. It contains a lot of solid information.

HARRY PRYSTOWSKY

BOOKS RECEIVED FOR REVIEW

Analyzing Psychotherapy. By SOLOMON KATZENELBOGEN. 126 pp., $3.00. Philosophical Library, New York, N. Y.

Clinical Orthopaedics, No. 11. Edited by ANTHONY F. DEPALMA. 257 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Diseases of the Esophagus. By J. TERRACOL AND RICHARD H. SWEET. 682 pp., $20.00. W. B. Saunders Co., Philadelphia, Pa.

Etiology and Treatment of Leukemia. Proceedings of the First Louisiana Cancer Conference. Edited by WALTER J. BURDETTE. 167 pp., $4.00. The C. V. Mosby Co., St. Louis, Mo.

A Handbook and Charting Manual for Student Nurses, 2nd edition. By ALICE L. PRICE. 279 pp., $4.50. The C. V. Mosby Co., St. Louis, Mo.

War Blinded Veterans in a Postwar Setting. A Social Work Followup of Rehabilitation Measures for Blinded Veterans with Service-Connected Disabilities Between Dec. 7, 1941 and Mar. 31, 1953. 260 pp., $1.50. U. S. Government Printing Office, Washington, D. C.

CHANGES IN REFLEX PATTERNS FOLLOWING SPINAL CORD HEMISECTION IN CATS[1, 2]

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In normal man at rest, electrical stimulation of posterior tibial nerve results in a characteristic pattern of action potentials in calf muscles (1, 2). With liminal stimuli, afferent fibers are excited and reflexly-produced deflections (H reflexes) make their appearance. Stronger shocks cause, in addition, M waves of shorter latency due to direct activation of motor axons (reference 2—Fig. 3). With even stronger stimuli, additional motor fibers are excited and antidromic impulses in these obliterate the H reflex discharge to muscle. Shocks of such blocking intensity are still well subliminal for many afferent fibers of mixed nerves which contribute to the reflex discharge (3). By this method, therefore, only a relatively small portion of the reflex responses to afferent impulses of lowest threshold can be sampled selectively in normal man. Reflex discharges from stronger stimuli can be detected, but only with full realization that they are influenced by antidromic volleys, and those resulting from impulses in afferent fibers of highest threshold cannot be studied by this means.

In man, however, rapid phasic stretch of muscle, as developed in tendon jerks, also results in afferent volleys in fibers of lowest threshold similar to those evoking H reflexes (1, 4). A component part of the H reflex recorded from muscle, and the tendon jerk as well, is due to monosynaptic discharge of motoneurons (2, 5, 6, 7). There are reasons, however, based on clinical experience and physiological studies in man, for suspecting that enhanced myotatic activity in patients with central neurological damage may be subserved by more complex connections within spinal cord (4). If that is correct, it is conceivable that, in normal man as well, myotatic reflexes other than monosynaptic may also result from rapid stretch of muscle or appropriate electrical stimulation of afferent fibers of lowest threshold. Due to experimental limitations already mentioned, it is difficult to investigate this possibility in man. For this reason, comparable and more definitive studies were undertaken in cats.

METHODS

Fifty-nine cats were used. Fourteen of these were perfectly normal at the onset of acute experiments which were carried out under anesthesia or high

[1] Supported by a grant (B-367) from The National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

[2] Part of this material was presented to the American Neurological Association in June 1956.

spinal cord transection. They will be described as "intact" cats. In 5 animals the spinal cord was hemisected during the course of acute study. In the remaining 40 animals aseptic spinal cord hemisection had been performed, at vertebral levels ranging from D4 to L1, three to seventy-four days prior to study. This operation will be called "chronic hemisection." None of these animals with chronic hemisection showed any evidence of urinary retention or other than unilateral weakness.

In all instances, reflex activity was investigated by using similarly-placed recording and stimulating electrodes on both sides of the body. The electrical stimuli, considered equal in intensity, were square waves of less than 0.5 msec. duration delivered alternately from the same stimulator. Under ether or pentobarbital (32 mgm./kg.) anesthesia, methods similar to those employed in man (2) were used. Stimuli were graded single shocks to tibial nerves at ankle, or in popliteal fossa proximal to the separation of nerves to gastrocnemius. These shocks were delivered through monopolar needles thrust through skin or, after dissection and freeing of the nerves, through paired platinum electrodes. Brisk mechanical taps to Achilles' tendon or sole of foot provided rapid stretch of calf muscles. Paired recording needle electrodes, insulated to the tips, were also pushed through skin into calf and small foot muscles. Action potentials were led from these electrodes and displayed, after standard amplification, on cathode ray beams and photographed.

Acute terminal experiments (6) were performed in some animals under pentobarbital anesthesia, but in the majority after transection of spinal cord at atlanto-occipital junction. In various instances, tibial nerve in popliteal fossa and at ankle, nerves to both heads of gastrocnemius, and sural nerve, were then exposed and freed, and stimulated directly through paired platinum electrodes. Resultant action potentials were recorded from muscles through needles or, after laminectomy, from proximal segments of severed L7 and S1 ventral roots through platinum electrodes.

RESULTS

I. Intact Animals

a) *Recording from Muscle*

1. Seven intact cats were *anesthetized* with pentobarbital, and 5 with ether. Single shocks, through needle electrodes, to tibial nerve in popliteal fossa failed to produce in any instance detectable H reflexes in calf muscles. With increasing strength of nerve stimulation motor volleys increased in size and effectively blocked all reflex discharge to those muscles. In all these animals, however, ankle jerks could be elicited and their action potentials recorded.

In the same 12 cats, the tibial nerve was similarly stimulated at the ankle.

In 5 animals (3 with pentobarbital and 2 with ether), only motor volleys could be detected in small foot muscles. In 3 animals, however, liminal shocks produced solely H reflexes and, in 4 more, H reflexes and motor volleys appeared at the same threshold of stimulation. These electrically-evoked reflex discharges to muscle were in all instances readily abolished with deeper planes of anesthesia despite persistence of ankle jerks.

2. Similar studies were made in animals after *acute high spinal transection*. In 15 cats the tibial nerve was stimulated in the popliteal fossa. In 4 of these animals H reflexes alone, with latencies of 9 to 10 milliseconds, were detected in calf muscles with liminal shocks (records 1, and 2, Fig. 1). With more intense stimuli (records 3 and 4, Fig. 1), motor fibers (M) were excited as well with consequent diminution of the recordable reflex discharge. In 7 additional cats, afferent impulses causing H reflexes and motor volleys had similar thresholds. In only 4 animals could motor volleys alone be detected from the muscle.

In 19 animals, tibial nerve was stimulated at the ankle and action potentials recorded from small foot muscles. In 10 of these cats weak shocks produced solely H reflex potentials with latencies varying from 10 to 11 milliseconds; in 6, reflex and motor deflections appeared at similar thresholds; in 3, no reflexes could be detected. In all these acutely spinal animals ankle jerks were readily detectable clinically and electromyographically.

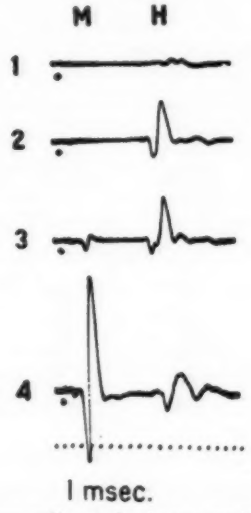

FIG. 1. Action potentials from calf muscles in cat made acutely spinal by transection at atlanto-occipital junction. Paired needle recording electrodes insulated to tips. Single shocks of increasing intensity to tibial nerve in popliteal fossa. Stimulus artefact in this and subsequent figures indicated by small dot. See text.

b) *Recording from Ventral Spinal Roots*

In each of 12 *acutely spinal* cats, some or all of the following peripheral nerves were carefully freed and stimulated directly by single shocks through paired platinum electrodes—sural nerve, tibial nerve in popliteal fossa and at ankle,

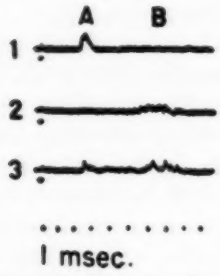

FIG. 2. Action potentials from proximal stump of severed L7 ventral roots in three different acutely spinal animals. Bipolar recording electrodes. Single liminal shocks to proximal portion of cut nerve to lateral head of gastrocnemius. See text.

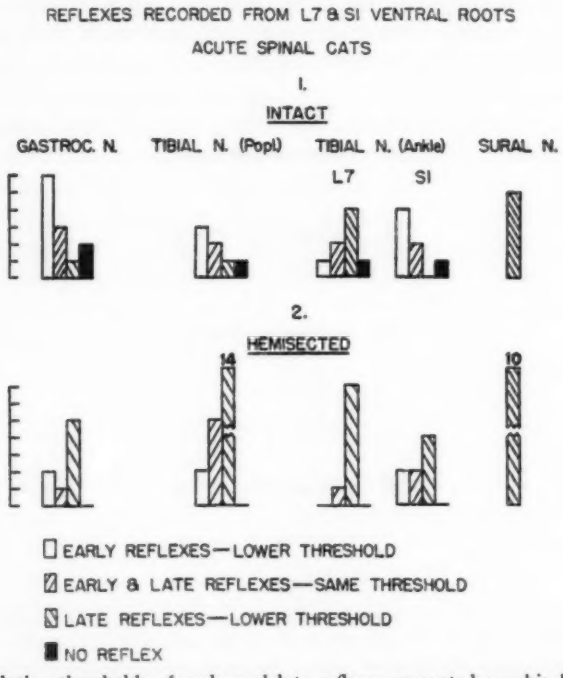

FIG. 3. Relative thresholds of early and late reflexes presented graphically. 1. Control animals. 2. Hemisected side in chronic animals.

and nerves to medial and lateral heads of gastrocnemius. Resultant reflex action potentials were recorded, after laminectomy, from proximal segments of the severed L7 and S1 ventral roots.

Sural nerve stimuli, regardless of intensity, evoked only repetitive deflections with latency of about 7 milliseconds. Similar shocks to all other nerves, however, produced two distinct reflex discharges, with latencies of 2 to 3 milliseconds, and 4 to 5 milliseconds, respectively, which we have called "early" and "late" reflexes. The relative thresholds of these early and late responses, however, differed not only in various animals, but also with the nerve which was stimulated and the root from which recordings were made (Figs. 2 and 3).

It can be seen from the upper histograms (1) of Figure 3 that, when the nerve to either head of gastrocnemius was stimulated, the results were similar regardless of whether L7 or S1 ventral root was used for recording. In 2 animals no reflex response was detected. In 4 cats, late reflexes appeared either at similar or lower thresholds than early responses. In the remaining 6 instances, however, liminal stimulation evoked only early discharges and increased shock intensity was necessary to recruit late reflexes. Similar results followed shocks to tibial nerve in popliteal fossa.

When, however, tibial nerve at ankle was the site of stimulation, the pattern was different as recorded from the two ventral roots. Early reflex discharge at lowest threshold was preponderant from S1 root, whereas liminal nerve stimulation caused mainly late reflex responses in L7 root.

II. Acute Hemisection of Spinal Cord

In 5 animals, made acutely spinal by high cervical cord transection, tibial nerves at ankles were stimulated by single shocks in the usual fashion. In 2 of these animals resultant action potentials were led from small foot muscles; in the remaining three, recording was from proximal segments of severed L7 and S1 ventral roots on the two sides. Subsequent acute hemisection of cord between D9 and D12 vertebral levels produced an immediate selective depression, or abolition, of late reflexes on the hemisected side. Threshold and amplitude of the early reflexes, however, remained unchanged from those recorded prior to hemisection, and similar to those on the intact side. This depression, following acute spinal hemisection, of late reflexes on the ipsilateral side was of a transient nature, lasting from a few minutes to half an hour.

III. Chronic Hemisection of Spinal Cord

Hemisection of spinal cord produced some permanent weakness of the ipsilateral hind limb. Immediately postoperatively, ankle jerks and muscle tone were likewise depressed on that side. Within a day or two however, in most instances, ankle jerks and tone both became clearly enhanced in the affected limb and remained so until sacrifice of the animal.

a) *Recording from Muscle*

1. In 5 cats under *pentobarbital anesthesia*, action potentials in small foot muscles resulting from stimulation of tibial nerve at ankle were recorded repeatedly from both hindlimbs. These studies were made prior to, and at different intervals after, aseptic hemisection of spinal cord. Muscle action potentials on the intact side remained unchanged from those in the preoperative state. On the hemisected side, however, the pattern was altered in a consistent fashion. The records of Figure 4 illustrate this change. Tracings in the left hand column show the effects of increasing strengths of nerve stimulation before hemisection. The low-threshold reflex deflection (A) of record 2 had a latent period of 10 milliseconds. This was little changed with increase in shock strength sufficient to excite some motor axons as well (record 3). With stronger stimuli (record 4), an additional deflection (B) with latency of 14 milliseconds also appeared, along with marked increase of the M wave. The tracings in the right hand column were similarly recorded six days after spinal cord hemisection at D11 vertebral level. In record 1, the least effective stimulus caused a small response after 16 milliseconds. With a stronger volley, as in record 2, latency of the polyphasic response (B) decreased to 13 milliseconds. Yet stronger shocks were needed (record 3) for emergence of the early spike (A) of 10 millisecond latency. In the final tracing more intense nerve stimulation, in addition to exciting many motor fibers, increased the amplitude of the early deflection at the expense of the later.

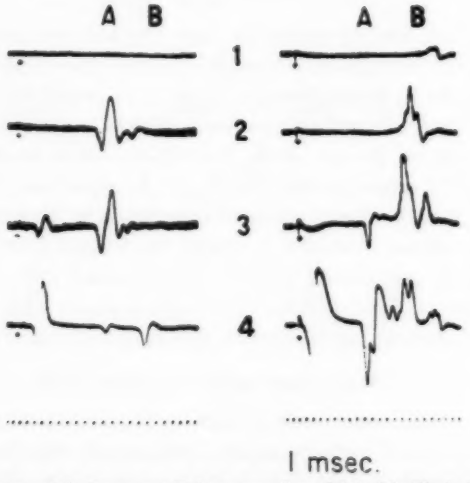

FIG. 4. Action potentials from small foot muscles of left hindlimb in cat under pentobarbital anesthesia. Left hand column—prior to spinal cord hemisection. Right hand column—6 days after section of left half of spinal cord (D7). Paired needle recording electrodes. Single shocks of increasing intensity to tibial nerve at ankle. See text.

In 3 of the remaining 4 animals similar results were encountered. Liminal nerve stimuli on the side of hemisection produced reflex deflections of long latencies ranging from 12 to 17 milliseconds. With stronger stimuli latencies decreased, and these late action potentials gained in size and duration, before the earlier deflections made their appearance. In the remaining cat, an early deflection with 10 millisecond latency appeared at lowest threshold. Slight increase in stimulus strength, however, brought in late responses which then grew disproportionately in size with increasingly stronger shocks.

2. Four animals, at intervals of 6 to 18 days after aseptic hemisection, were made *acutely spinal* by transection at atlanto-occipital junction. In these animals, reflex deflections of long latency only appeared, on the intact side, with stimulus strengths considerably greater than those necessary to cause early reflex discharge and considerable direct motor nerve excitation. On the hemisected side, in contrast, late deflections of long latency appeared at lowest threshold in 3 cats, and increased disproportionately with greater stimulus strength.

b) *Recording from Ventral Roots*

1. Eight cats, at intervals of 8 to 22 days after hemisection, were made *acutely spinal* at C1 level. Action potentials were recorded from the proximal stumps of L7 and S1 ventral roots on both sides. Though it was clearly impossible to assure completely comparable stimuli on both sides, thresholds to reflex discharge from light taps on Achilles' tendon, whether judged clinically or from action potentials, were obviously lower on the hemisected side. With the weakest tendon taps, latency of the reflex responses on that side ranged from 7 to 10 milliseconds. On the intact side, in contrast, the reflex discharge of lowest threshold appeared after 4 to 6 milliseconds. With more vigorous taps, this difference in latency between the two sides became obscured; additional early deflections then appeared on the hemisected side as well. In all instances however, with these stronger blows, the greater size and duration of the reflex response on the hemisected side was in the later deflections. Representative records illustrating these differences in threshold and configuration are shown in Figure 5.

2. Twenty-one animals, with spinal cord hemisected from 5 to 74 days previously, were made *acutely spinal* by transection at the atlanto-occipital junction. In these cats, tibial nerves were freed bilaterally in popliteal fossa, or at ankle, and stimulated directly. Action potentials were recorded from proximal portions of severed L7 and S1 ventral roots. In 19 of these cats weak nerve shocks, totally ineffective on the intact side, produced reflex discharge on the side of hemisection. Latencies ranged from 4 to 6 milliseconds, dependent partly on site of nerve stimulation. Stronger stimuli caused, in addition to enhanced size of this response, early deflections with latencies varying from 2

to 3 milliseconds. Such stimuli also produced, but at this higher threshold, early and late deflections on the intact side. Indeed, thresholds for early reflexes were essentially similar on the two sides of the body. With increasing shock intensity the late deflections were always of greater amplitude on the hemisected side. In contrast, in all but 3 animals, the early potentials reached greater size on the intact side. The pattern is illustrated in the records of Figure 6. The tracings in the right hand column were recorded from the hemisected side, those on the left were from the intact side. Weakest shocks, in records 3, produced only late reflex discharge (B), with latency of 5 millisec-

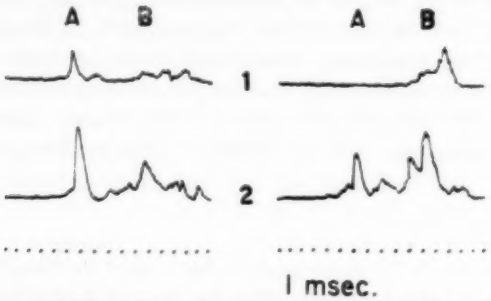

FIG. 5. Action potentials from L7 ventral roots in cat with spinal cord hemisection (D8) eight days previously. Acute spinal preparation. Left hand records—intact side. Right hand records—hemisected side. Tracings 1 and 2 show, respectively, stretch reflex responses to weak and stronger taps to Achilles' tendon. Sweeps of cathode ray beam triggered by tap. See text.

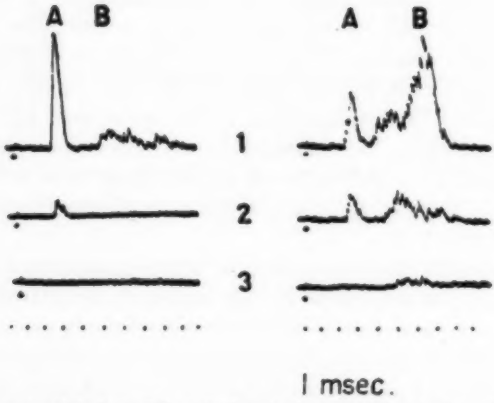

FIG. 6. Action potentials from L7 ventral roots in cat with spinal cord hemisection (D8) one month previously. Acute spinal preparation. Left hand column—intact side. Right hand column—hemisected side. Single shocks of decreasing intensity to proximal portion of severed tibial nerve in popliteal fossa. See text.

onds, on the hemisected side. Slightly stronger stimuli, as in records 2, caused an additional early deflection (A) of 2 millisecond latency on that side, and a similar early potential on the intact side as well. With stronger nerve shocks (records 1), the increased amplitude was greater in the later deflections on the hemisected side, and in the early spike potential on the intact side.

3. In 8 cats, with upper cervical *spinal cord similarly transected* 5 to 46 days after hemisection, nerves to medial and lateral heads of gastrocnemius were freed in both hindlimbs and stimulated electrically by single shocks of varying strengths. The resultant action potentials differed considerably on the two sides, though unilateral differences between records from L7 and S1 ventral roots were not appreciable.

In 5 of these animals, weak nerve shocks produced reflex discharge on the hemisected side, when similar stimuli were ineffective on the intact side. Moreover, these deflections of lowest threshold on the side of hemisection were polyphasic, with latencies of about 5 milliseconds. With somewhat stronger nerve stimulation, earlier spike discharges of 2 millisecond latency appeared at approximately the same threshold on both sides. Further increase in stimulus strength produced more early discharge on the intact side with comparatively meager late response. On the hemisected side, in contrast, reflex enhancement with increasing strengths of nerve shock was manifested mainly in greater size and duration of the later deflections. Indeed, early spike reflexes on the hemisected side, regardless of stimulus strength, never achieved the size of those on the intact side. These findings are illustrated in the records of Figure 7. On the hemisected side (right hand column), only late reflex discharge (B) appeared with liminal stimuli (record 4). Similar nerve shocks on the intact side were ineffective. Stronger afferent volleys, in records 3, caused in addition early deflections (A) on both sides. With further increase in stimulus intensity (records 2 and 1), the late potentials grew markedly in amplitude and duration on the hemisected side although early spikes (A) never reached the size of those on the intact side. Late deflections (B), on the contrary, only became evident on the intact side with relatively strong volleys (record 1).

In the remaining 3 animals, no difference in reflex threshold could be detected between the two sides. Early and late deflections appeared similarly with liminal stimuli. Nerve volleys of increasing intensity, however, always caused early deflections of considerably greater amplitude on the intact side, and enhanced late discharges on the hemisected side.

4. In 10 cats with chronic spinal hemisection, similar experiments in the *acutely spinal state* were performed with sural nerve stimulation. No difference in reflex threshold could be detected between the sides. In 3 animals, amplitude and configuration on the two sides were not appreciably different. In the remaining 7 cats, the reflexes were of greater size and duration on the side of

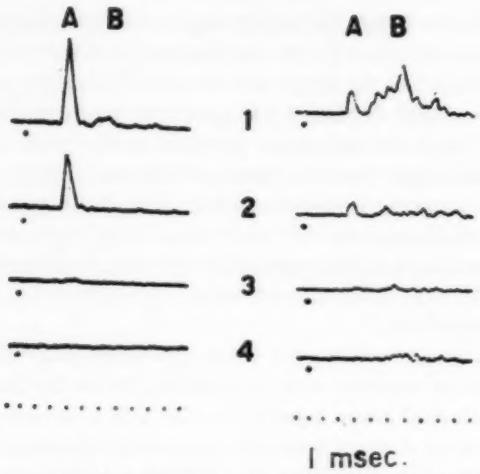

FIG. 7. Action potentials from L7 ventral roots in cat with spinal cord hemisection (D8) three weeks previously. Acute spinal preparation. Left hand column—intact side. Right hand column—hemisected side. Single shocks of decreasing intensity to proximal portion of severed nerve to lateral head of gastrocnemius. See text.

hemisection. The difference, however, was considerably less than that encountered (see above) with mixed and muscle nerve stimulation.

For clarity and in summary, the reflex responses on the hemisected sides of all animals studied have been plotted in the lower histogram (2) of Figure 3, in a manner similar to that used for intact cats. It can be seen, in contrast to the results with intact animals, and regardless of nerve or site of stimulation, that the reflex discharge of lowest threshold from ventral roots was, with few exceptions, of the "late" variety.

DISCUSSION

In anesthetized intact cats, single shocks to tibial nerves rarely cause detectable H reflex discharge to muscle at thresholds below those of motor axons. In acutely spinal preparations, liminal stimuli produce low-threshold reflexes in about half of animals and, in most of the remainder, the thresholds of reflex and motor responses are the same. Even under these more favorable conditions, however, the detectable reflex responses to muscle are readily abolished by antidromic volleys resulting from stronger stimuli. The portion, therefore, of reflex discharge by impulses in afferent fibers of lowest threshold, which can be detected by this method, is considerably smaller than that encountered in human studies (2). Reasons for this experimental discrepancy are not clear. The much greater size of nerve trunks in man may permit selective stimulation of larger bundles of afferent fibers than is possible in cats. This is not, however,

complete explanation of the discrepancy since, in cats, anesthesia and high spinal cord transection have differing effects on the size of the detectable portion of the reflex response. This may well suggest that these experimental procedures, acute spinal transection and anesthesia, have in themselves inhibitory influence on reflex discharge at lowest threshold. Finally, it remains quite possible, due to species differences, that spinal motoneuron pools may be fired reflexly in man more effectively than in cats by afferent volleys of lowest threshold.

Despite this dissimilarity it is evident, particularly in acutely spinal preparations, that physiological features underlying H reflex responses can be studied similarly in cats, and delineated in some respects more precisely than in man. When, for example, in intact cats the reflex responses of shocks at Group I strength to tibial nerve are recorded from proximal stumps of severed ventral roots, two discharges—"early" and "late" are regularly detected at some stimulus intensity or another. The early sharp spikes are clearly those resulting from monosynaptic discharge of motoneurons (5, 6, 8). The later repetitive potentials have likewise been identified as polysynaptic responses (5, 6, 9, 10). In a few cats, only these polysynaptic potentials of long latency appear at lowest threshold (Fig. 3). In most, however, monosynaptic spikes result from liminal shocks. When monosynaptic responses are great, polysynaptic deflections are not prominent; conversely poorly expressed monosynaptic discharge is often followed by well developed polysynaptic potentials. This reciprocal alternation has also been noted by others (9, 10). Undoubtedly a portion of the polysynaptic reflex response is due to activation of large afferent fibers, in tibial nerve, from skin. That most, however, cannot be so explained on cutaneous sources is suggested by the observations that similar polysynaptic deflections appear, and in some animals at lowest threshold, when purely muscle nerves are stimulated.

Evidence of this low-threshold polysynaptic myotatic component is not, however, detectable in action potentials accompanying ankle jerks in intact animals. Latencies of reflex responses to weak taps are little different from those resulting from stronger blows. Apparently, then, with this relatively crude means of muscle stretch, the first-appearing monosynaptic component dominates the picture regardless of intensity of stimulation. When, however, under deep planes of pentobarbital or ether anesthesia, low-threshold reflex responses to nerve shocks are abolished, ankle jerks are retained. Under these circumstances, reflex mechanisms of higher threshold are seemingly active.

This division of H reflexes into monosynaptic and polysynaptic components is exaggerated after spinal cord hemisection. Immediately after hemisection, there is transient marked depression of polysynaptic activity on the side of the lesion. Within a few days after operation, however, the converse becomes true (Fig. 3). Whether recorded from muscle in the manner employed in man,

or from ventral roots, the threshold to reflex response is lower on the hemisected side and the potentials with liminal nerve stimuli are of long latency. This is not surprising since action potentials of the exaggerated ankle jerks on that hemisected side have also, with weakest taps, increased latency. Only with stronger shocks, or heavier blows, do additional monosynaptic responses of shorter latency make their appearance. Moreover, this increased polysynaptic reflex excitability on the hemisected side is expressed, not only by different threshold and latency, but also, with increasingly stronger stimuli, by enhanced size and duration of response. Again, the role of cutaneous afferent fibers must be insignificant since the results of stimuli to muscle nerves differ little from those to mixed nerve trunks, and polysynaptic discharge from sural nerve stimulation is increased proportionately less.

It seems, therefore, that after spinal cord hemisection in cats the pattern of myotatic activity changes. Enhanced reflex response is largely characterized by increased excitability within polysynaptic arcs. No doubt part of this increased excitability, when strong stimuli are employed, is more apparent than real. Certainly monosynaptic discharge is rarely as complete under these conditions as normally, and thus fewer motoneurons are pre-empted by that mechanism. With weaker stimuli, however, it is evident that the lowered threshold for polysynaptic discharge on that side is real, despite the fact that monosynaptic thresholds are little different on the two sides.

Mechanisms underlying this altered myotatic reflex pattern in cats by prior chronic spinal cord hemisection remain obscure. It has been suggested (10) that, under these conditions, the changed reflex pattern reflects plasticity of intraspinal organization whereby new mechanisms supplant others which have become less effective. Indeed, there is some anatomical evidence (11) to suggest that, two or three weeks after hemisection, actual sprouting of dorsal root fibers occurs and they may, at that stage, form new synaptic connections. In the present studies, the fact that monosynaptic discharge was rarely as complete on the side of spinal hemisection as on the normal side might also lend certain support to that thesis. The physiological alterations herein described, however, occurred within a few days of hemisection and thus long before structural alterations (11) take place. Moreover, only the threshold for polysynaptic reflex discharge was altered. That to monosynaptic firing was unchanged. Apparently, then, some selective change takes place at interneuronal level. This change need not necessarily result in new myotatic arcs. It might merely, and so seems likely to the present authors, accentuate polysynaptic portions of normally multiple reflex responses. Such would, in fact, be anticipated if the alteration were due to interruption of pyramidal pathways which normally in cats terminate at interneuronal, rather than motoneuronal, level (12). It might be that the results of this interruption are due merely to removal of normal inhibitory influences, exerted through these de-

scending pathways, on polysynaptic reflex mechanisms. Equally well, however, they may be due to increased sensitivity of the participating interneurons similar to that revealed by pharmacological means (13).

SUMMARY

Spinal reflexes resulting from nerve stimulation and tendon tap have been studied bilaterally in intact cats, and in cats at various intervals after spinal cord hemisection.

H reflexes to muscle, and tendon jerks, have been shown normally to have monosynaptic and polysynaptic components as detected in reflexly discharged action potentials led from ventral roots and muscle.

Immediately after spinal cord hemisection, polysynaptic reflexes are depressed transiently on the side of hemisection. Within a few days thereafter, thresholds of polysynaptic reflex discharge are markedly lowered on the hemisected side, although those of monosynaptic responses are little altered.

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STRETCH REFLEXES IN PATIENTS WITH SPINAL CORD LESIONS[1]

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In patients with upper motor neuron damage due to lesions of cerebral hemisphere or high brain-stem, spinal motoneuron excitability as tested by low-threshold afferent nerve volleys is little different from that in normal persons (1). When, however, similar damage occurs at low brain-stem or spinal cord level, the threshold for reflex motoneuron discharge is greatly lowered (2). Indeed, under those circumstances, H reflexes evoked by single nerve shocks are readily detectable in all accessible muscles. Since some components, at least, of H reflexes are monosynaptic (3), it was assumed that the enhanced reflex response to electrically-excited afferent volleys found in such patients resulted from increased excitability within two-neuron arcs (2). This assumption seemed reasonable since it was considered that variations in wave form and latency of muscle action potentials, which are of several milliseconds duration, were due to indiscriminate recording from multiple groups of muscle units.

Since those observations were made in man, however, the validity of the assumption has been questioned by the results of comparable studies in cats with chronic spinal cord hemisection (4). In those animals the lowered threshold of reflex response, present on the hemisected side, is due, not to enhanced monosynaptic discharge but to more effective polysynaptic excitation of motoneurons by low-threshold afferent nerve volleys from muscle. This is the case regardless of whether the afferent volleys result from electrical stimuli to nerve or from tendon tap. All records of muscle action potentials from man which formed the basis of the previous studies (1, 2) have therefore been re-examined with particular attention to the wave form and latency of those deflections which appeared at lowest threshold. The object was to determine whether distinctive features characterize the records of patients, as well as cats, with chronic spinal cord lesions. The experimental methods have been described in detail previously (1).

RESULTS

Latency of H Reflexes and Ankle Jerks

I. In normal persons and patients with hemispheral or high brain-stem lesions

There were 26 normal persons and 18 patients with upper motor neuron damage identified clinically as due to lesions at hemispheral or high brain-

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stem level. The records of eight of these were of necessity discarded because speed of recording or intensity of nerve stimulation were inadequate for accurate measurement of latency and configuration of the reflex response which appeared at lowest threshold. In the remaining thirty-six, the action potentials evoked reflexly by just supraliminal nerve shocks had latent periods which did not vary beyond the limits of *30 and 32 milliseconds*. With stronger nerve volleys, this latency was unaltered—the initial deflection increased in amplitude with or without the addition of succeeding polyphasic waves. With tendon taps, less susceptible to precise grading of intensity, the latent periods showed somewhat greater spread between *35 and 38 milliseconds*. The longer latencies were usually reduced to the shorter values when heavier blows were employed.

II. In patients with spinal cord or low brain-stem lesions

The records of 27 patients with chronic lesions of spinal cord or low brainstem were suitable for analysis. In these patients, the configuration of the reflexly-evoked muscle action potentials was distinctive and followed one of two patterns.

In most instances, as shown in the records of Figure 1, weakest effective afferent nerve stimulation produced two reflex deflections (record 1). The first potential (A) had a latency of *30 milliseconds*. It was followed by a second, more prominent, deflection (B) with a latent period of *38 milliseconds*. With stronger nerve shocks (records 2 and 3), both deflections gained in amplitude. This increase, however, was always more marked with the later (B) potential.

Less commonly, as illustrated by Figure 2, liminal nerve shocks caused reflex discharge to muscle only after long latent periods. In records 1 and 2, for example, the latency (B) was *40 milliseconds*. Stronger nerve volleys, as in

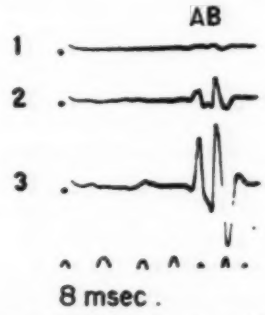

FIG. 1. Action potentials from calf muscles of patient with spinal cord lesion at 6th thoracic segmental level. Surface electrodes. Single shocks of increasing intensity to posterior tibial nerve in popliteal fossa. Stimulus artefact in this and following figure indicated by small dot.

records 3 and 4, caused additional earlier deflections (A) of *32 millisecond* latency.

In 7 of these patients, action potentials from calf muscles elicited by tapping Achilles' tendon were also available for study. In one patient, latencies of the reflex responses to weakest taps ranged from 35 to 38 milliseconds. In all others, with comparable light taps, the latent periods were always *greater than 40 milliseconds*. More intense blows to Achilles' tendon caused appreciable shortening of latency. The pattern is illustrated in Figure 3. In record 5, a liminal tap produced deflection B after a latency of 44 milliseconds. A somewhat stronger blow (record 3) caused an additional earlier potential of shorter latent period. In records 2 and 1, more intense stimuli resulted in further decreased latency to 36 milliseconds, and increased size of early and late deflections.

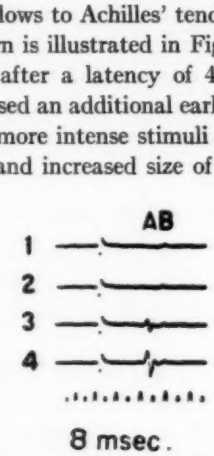

FIG. 2. Action potentials from calf muscles of patient with cervical spinal cord damage due to syringomyelia. Surface electrodes. Single shocks of increasing intensity to posterior tibial nerve in popliteal fossa.

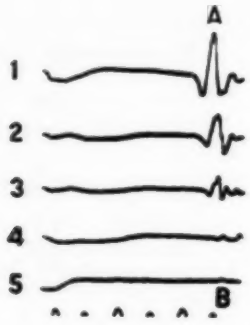

FIG. 3. Action potentials from calf muscles of patient with mid-thoracic spinal cord lesion. Surface electrodes. Records in sequence show stretch reflex response produced by successively weaker taps to Achilles' tendon. Sweep of cathode ray beam initiated by hammer blow.

DISCUSSION

Increased myotatic activity, expressed by enhanced briskness and lowered threshold of tendon jerks, is common clinically in patients with upper motor neuron damage. When the lesions occur in lower brain-stem or spinal cord, thresholds of electrically-evoked H reflexes are also markedly lowered (2). In such patients, damage is usually bilateral and thus comparison of reflex activity between sides is impossible. Normal persons or patients with hemispheral lesions may, however, be justifiably accepted as control subjects. When that was done in the present study, it is apparent that in the control group latencies of the muscle action potentials accompanying minimally-detectable H reflexes and ankle jerks were remarkably constant. They varied between 30 to 32, and 35 to 38, milliseconds respectively. Moreover, when stronger stimuli, electrical or mechanical, were employed there was little change in these values. In contrast, the results were different in patients with low brain-stem or spinal cord lesions. In some instances latencies of the muscle action potentials of H reflexes produced by weakest effective stimuli, and in all but one patient those of tendon jerks, were found considerably longer by about 6 milliseconds. In the remaining patients, two components—early and late, separated by about 6 milliseconds—resulted from liminal stimulation. In all, stronger stimuli produced composite action potentials with shortest latency within ranges found in control subjects. With reference to the late reflex discharge, therefore, the electromyographic findings in these patients with spinal or low brain-stem lesions were similar to those in cats with chronically hemisected spinal cords (4). By analogy, therefore, it seems reasonable to infer that, in these patients, polysynaptic myotatic mechanisms were likewise enhanced.

In this group of patients, however, H reflexes and tendon jerks, when due to stimuli sufficiently intense that latencies were shortened to normal values, had also thresholds lower than those in control subjects (2). It is evident, therefore, that in humans, as opposed to cats, the increased reflex activity with spinal cord lesions applies to monosynaptic as well as to polysynaptic arcs. Reasons have already been suggested (2) for assuming that the responsible damage is to motor pathways descending from brain-stem and possibly through medullary pyramids. This species difference between cat and man is perhaps to be anticipated on anatomical and physiological grounds. It seems clear that if, in cats, descending motor fibers terminate directly on spinal motoneurons, the numbers must be few (5, 6). Virtually all end on interneurons. In monkeys, however, additional direct synaptic connections with motoneurons occur (7, 8, 9). Also, in man, there is fairly convincing physiological evidence of similar direct termination on motor nerve cells as well as on intermediate neurons (10). In humans, therefore, and probably other primates, it appears that both

motoneurons and interneurons are affected by damage, in spinal cord or lower brain-stem, of descending motor pathways. The resultant lowered threshold to reflex discharge in man is thus a function of increased excitability in more than one myotatic reflex mechanism.

These observations in man and cats with chronic lesions of the spinal cord indicate strongly, therefore, a differentiation of multiple myotatic processes. This would certainly allow interpretations of some clinical investigative significance. It is reasonable to infer that this fractionation of reflex response, under these pathological conditions, represents merely potentiation of reflex processes which are normally operative. H reflexes recorded from muscle, in contrast to a previous interpretation (3), must probably be regarded in normal man, as well as in patients with spinal cord or low brain-stem lesions, as monosynaptic and also polysynaptic responses to afferent volleys of lowest threshold. Whether or not tendon jerks employ, in addition to these mechanisms, myotatic reflexes of higher threshold (4), or merely differ from H reflexes in gamma efferent control of their receptor organs (12), is not known.

SUMMARY

Latencies of muscle action potentials accompanying H reflexes and tendon jerks have been compared in control persons and patients with upper motor neuron damage due to lesions in spinal cord or low brain-stem.

In ankle jerks resulting from light taps in all but one patient, and liminal H reflex discharges in some, latencies were greater, by several milliseconds, in the patients with spinal cord or low brain-stem damage. Stronger stimulation shortened latent periods to normal values by the addition of earlier deflections. In the remaining patients, early and late deflections resulted from liminal stimulation and increased proportionately with stronger stimuli. The lower threshold to effective reflex response in these patients is due to enhancement of both monosynaptic and polysynaptic mechanisms.

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OXYGEN TENSION AND ACID-BASE ADJUSTMENT IN CARDIAC FAILURE

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Two striking changes may occur in the arterial blood during left ventricular failure: a lowering of the oxyhemoglobin saturation, and the development of respiratory alkalosis (1). These changes are closely related to the ventilation and perfusion of the lungs (2). When left ventricular failure occurs in a patient with severe obstructive emphysema, a respiratory acidosis similar to that seen when pneumonia is associated with emphysema (3) may develop. This paper deals with the physiologic mechanisms and treatment of some of these alterations.

Acid-base changes, basal metabolism and ventilation measurements in patients with heart disease were reported in a series of papers between 1914 and 1917 (4, 5) by Francis Peabody, but suffered from the lack of arterial blood studies. It was not until Stadie proved arterial puncture to be safe around 1919 (6) that a technique was available to study arterial blood directly. Haldane and Barcroft had already devised simplified methods for determining blood gases, but after 1924, Van Slyke's method was widely used (7).

Harrop (8) in 1919 and Meakins (9) studied both arterial and venous blood during heart failure, showing that there was frequently decreased oxygenation of the venous blood. Although the majority of patients in left ventricular failure show a normal degree of arterial saturation, and a normal or low carbon dioxide content, certain cases were observed in which there was carbon dioxide retention (10).

Because the acid-base balance has not been studied seriatum during recovery from heart failure and because new concepts of distribution and perfusion of the lungs (11, 12) have not been applied to patients in heart failure, the present study was undertaken.

Methods: All studies of ventilation and oxygen consumption were done in the post-absorptive state after at least one-half hour of rest in bed. The arterial blood was analyzed for oxygen and carbon dioxide pressure by the method of Riley, *et al.* (13). We consider this method more accurate in demonstrating small changes in the degree of oxygenation of arterial blood than the determination of saturation. This is particularly true above 90 per cent saturation where the oxygen dissociation curve is nearly flat.

Carbon dixoide content was determined by the method of Van Slyke and Neill (7). The pH was estimated by the use of the nomogram of Singer and Hastings (14). Expired air was collected in a Tissot spirometer and analyzed by the method of Scholander (15).

Material: Thirty-seven patients with heart disease in various degrees of heart failure were studied. Some were studied only once, others repeatedly. The patients were then divided into four groups.

Group I. All patients in this group had basilar rales at the time of at least one study and no clinical evidence of pulmonary emphysema. There were six such patients (Cases 1–6). Most of them had hypertensive cardiovascular disease or arteriosclerotic heart disease. One had syphilitic aortic insufficiency. This group is made up of patients in mild to moderate left ventricular failure and includes no patients with severe pulmonary edema at the time of study (Chart 1). Some of these patients were studied after pulmonary rales had disappeared, and these studies are included to show the pattern of changes with improvement in cardiac compensation.

Group II. All patients in this group were in compensated cardiac failure to the extent that none had basilar rales at the time of study. The diagnoses in this group were varied. There were patients with arteriosclerotic heart disease, myocardial infarction, myocarditis, syphilitic aortic insufficiency and rheumatic heart disease. There were fifteen patients (Cases 7–21). A few had some evidence of right ventricular failure (Chart 2).

Group III. This group is made up of three patients (Cases 22, 23 and 24) who had acute and very severe pulmonary edema at the time of study. Two died on the day of study, and severe pulmonary edema was confirmed at autopsy in one patient. All had myocardial infarctions either old or recent (Chart 3).

Group IV. This group consists of thirteen patients (Cases 25 to 37) all of whom had clinical evidence of pulmonary emphysema, and, in addition, arteriosclerotic heart disease, hypertensive cardiovascular disease, rheumatic heart disease or non-specific myocarditis. All patients in this group showed either an enlarged heart, hypertension, electrocardiographic or autopsy evidence of left ventricular heart disease (Chart 4).

Results: Group I: The general pattern of the acid-base findings is demonstrated in Chart 5. With few exceptions patients tended to have a low arterial blood carbon dioxide pressure (P_{CO_2}) and arterial blood carbon dioxide content (C_{CO_2}) with respiratory alkalosis when basilar rales were heard in the lungs. The arterial blood oxyhemoglobin saturation tended to be just below normal limits. The arterial blood oxygen tension (P_{O_2}) was usually markedly depressed. Except when initial P_{O_2} was very low, there was little tendency for P_{O_2} to rise with improved compensation. Where ventilatory and oxygen consumption studies were done, both were well above normal figures.

With improvement in cardiac compensation, there was tendency for the pH to return toward normal, but this change did not occur universally. The most consistent change with clearing of the lungs of rales and loss of edema was a drop in ventilation and oxygen consumption.

Certain exceptions to the above generalizations will be noted in Chart 5. In Case 1, the pH was low normal on the first study. The C_{CO_2}, however, was low and P_{CO_2} was normal. The study was performed late in the afternoon. The patient had been ambulatory in the outpatient clinic during the entire day. The study was done after one-half hour's bed rest. The explanation appears to be that while the patient was ambulatory, hyperventilation was marked. It was associated with a low P_{CO_2}, respiratory alkalosis and a com-

CHART 1

*Clinical Data, Diagnosis and Autopsy Findings on Patients in Group I**

	Patient, Age, Color, Sex, Hist. #, Admission Date	Heart Size	Heart Size by X-ray	E.C.G.	Diagnosis	Date of Death	Autopsy Finding
1	T.P. 64 WM 176375 11-5-53	++	++	P.M.I.O.	H.C.V.D. A.S.H.D.	10-24-56	No Autopsy
2	C.B. 55 CM 175430 10-6-53	++	++	L.V.H.	A.S.H.D.		
3	C.M. 71 WM 181698 4-12-54 10-14-55	++	++	S.T.	A.S.H.D.		
4	A.B. 67 CM 193814 2-15-55	—	+++	L.B.B.B.	S.A.I.	2-16-55	No Autopsy
5	D.L. 38 WM 182407 5-5-54	N	—	A.M.I.A. 5-5-54	A.S.H.D.		
6	M.G. 64 WF 179787 2-15-54	++	++	P.A.T. 2-15-54	H.C.V.D. A.S.H.D. P.A.T.	2-16-54	No Autopsy

* Key to charts:

A.E. = Ankle Edema
\+ = Mild
++ = Moderate
+++ = Severe
++++ = Massive

Digitalis

S = Started but incomplete
C = Digitalization just completed
M = Maintenance

E.H. = Enlarged Heart
\+ = Slight enlargement
++ = Moderate enlargement
+++ = Marked enlargement
– = Not Recorded

Electrocardiogram

L.V. = Low Voltage
P.M.I.O. = Posterior Myocardial Infarction, Old
P.M.I.A. = Posterior Myocardial Infarction, Acute
A.M.I.O. = Anterior Myocardial Infarction, Old
S.T. = Non-specific ST and T wave changes
L.V.H. = Left Ventricular Hypertrophy
P.A.T. = Paroxysmal Auricular Tachycardia
D.P.T. = Delayed Precordial Transition
L.B.B.B. = Left Bundle Branch Block
A.F. = Auricular Fibrillation
N. = Normal

Diagnosis

H.C.V.D. = Hypertensive Cardiovascular Disease
A.S.H.D. = Arteriosclerotic Heart Disease
R.H.D. = Rheumatic Heart Disease
M.I. = Mitral Insufficiency
M.S. = Mitral Stenosis
A.S. = Aortic Stenosis
S.A.I. = Syphilitic Aortic Insufficiency
M. = Myocarditis
P.E. = Pulmonary Edema
O.E. = Obstructive Emphysema
H.A. = History of Asthma
L.E. = Pulmonary Embolus
B.E. = Brain Embolus
P.B. = Periodic Breathing

CHART 2

Clinical Data and Diagnosis and Autopsy Findings

	Patient, Age. Color, Sex, Hist. #, Admission Date	Heart Size	Heart Size by X-ray	E.C.G.	Diagnosis	Date of Death	Autopsy Finding
7	J.H. 70 WM 195997 4-6-55	+	+++	A.M.I.O.	H.C.V.D.	4-9-55	a) Arteriosclerosis of the Coronary Arteries. b) Acute Myocardial Necrosis Posterior Wall, Left Ventricle; c) Pulmonary Edema; d) Pulmonary Emphysema, mild.
8	J.S. 49 CF 178947 1-20-54	+++	+++	S.T.	R.H.D. M.I. A.S.		
9	A.P. 53 WM 180113 2-24-54	+	+	A.M.I.A. 2-24-54	A.S.H.D. B.E. 3-15-54		
10	L.J. 39 CF 183066 5-26-54	+	+	S.T. A.F.	R.H.D. M.I. M.S.		
11	L.K. 55 WM 181316 3-31-54	N	—	P.M.I.A. 3-31-54	A.S.H.D.	4-9-54	No Autopsy
12	J.R. 38 WM 184752 7-12-54	N	+	P.M.I.A. 7-12-54	A.S.H.D.		
13	L.R. 63 WM 181672 4-12-54	+	+	A.M.I.A. 4-12-54	A.S.H.D. H.C.V.D.		
14	E.P. 50 CM 187194 9-8-54	+++	+++	S.T.	S.A.I.	9-18-55	No Autopsy
15	A.K. 66 WM 200063 7-11-55	+	—	P.M.I.A. 7-11-57	A.S.H.D.	7-14-55	a) Acute Thrombosis Left Circumflex Coronary; b) Acute Myocardial Necrosis Posterior Wall, Left Ventricle; c) Pulmonary Emphysema.
16	C.R. 37 CM 197028 5-2-55	++	+++	L.V.H.	M.		
17	R.J. 67 CF 215950 7-16-56	+++	+++	A.F. S.T.	A.S.H.D.		
18	M.W. 34 WF 179102 1-25-54	N	N	N	R.H.D. M.S.		
19	A.W. 23 CF 189782 11-10-54	+	+	D.P.T.	R.H.D. M.S.		
20	J.D. 17 WF 228893 6-30-55	N	N	R.V.H.	R.H.D. M.S.		
21	M.H. 181385 8-19-54	N	N	N	R.H.D. M.S.		

CHART 3

Clinical Data, Diagnosis and Autopsy Findings in Group III

	Patient, Age, Color, Sex, Hist. #, Admission Date	Heart Size	Heart Size by X-ray	E.C.G.	Diagnosis	Date of Death	Autopsy Finding
22	G.L. 71 WF 175658 10-13-55 5-3-55	N	+	A.M.I.O.	H.C.V.D.		
23	C.C. 78 WM 180871 3-18-54	—	+	A.M.I.A. 3-18-54	A.S.H.D.	3-18-54	No Autopsy
24	B.D. 42 WM 188412 10-7-54	—	—	—	H.C.V.D. H.A.	10-7-54	a) Thrombus Anterior Descending Branch, Left Coronary Artery; b) Myocardial Infarction Healed, Anterior Septum; c) Acute Pulmonary Edema.

pensatory lowering of the C_{CO_2}. When the patient was allowed to rest in bed, ventilation decreased, and the P_{CO_2} returned to normal. The C_{CO_2}, however, was regulated more slowly, resulting in a low normal pH.

In Case 3 the production of a rather marked respiratory alkalosis by an acute pulmonary embolus is illustrated. Ammonium chloride apparently had little effect on the acid-base balance in Case 1, but in Case 2, when it was first given, a moderate metabolic acidosis resulted.

This group differs from Group II in the fact that a study happened to have been carried out while the patient had moist rales at the bases. The studies in Group I demonstrate serial changes occurring in the blood with clinical improvement in heart failure. In Group II no patients had pulmonary rales at the times of study. Combining Group I and II we can average the values of individual studies where the patients showed basilar rales, and when the lungs were clear. Chart 9 shows the mean values in the different studies of patients in mild pulmonary edema (Group I), patients with no signs of pulmonary edema, (Groups I and II), acute massive pulmonary edema (Group III) and heart failure with pulmonary emphysema (Group IV).

The histories on several of the patients whose studies are depicted in Chart 5 follow:

Case 1: T.P. (B.C.H. #176375) a 64 year old Norwegian seaman entered the Baltimore City Hospitals on November 5, 1953 complaining of severe dyspnea and ankle swelling.

About six months prior to admission he first noted shortness of breath on exertion. The dyspnea increased, and later orthopnea, paroxysmal nocturnal dyspnea, ankle and adbominal swelling developed. There had been no chest pain.

Examination showed a temperature of 100° F., a pulse rate of 100, respirations of 30, and a blood pressure of 170/110. He was extremely dyspneic and orthopneic. The neck

CHART 4

Clinical Data, Diagnosis and Autopsy Findings in Group IV

	Patient, Age, Color, Sex, Hist. #, Admission Date	Heart Size	Heart Size by X-ray	E.C.G.	Diagnosis	Date of Death	Autopsy Findings
25	J.K. 50 WM 183249 5-31-54	N	—	P.M.I.A. 5-31-54	O.E. A.S.H.D.		
26	C.W. 47 WM 215783 7-12-56	++	+++	L.B.B.B.	O.E. M.	9-8-56	a) Diffuse non-specific Myocarditis; b) Pulmonary Emphysema.
27	M.D. 59 WM 19691 3-18-54	+++	+++	S.T.	O.E. R.H.D. A.S.	3-23-54	No Autopsy
28	J.S. 85 WM 174888 10-21-53	N	N	P.M.I.A. 10-21-53	A.S.H.D. O.E.		
29	M.K. 52 WF 178751 1-15-54	++	−	A.M.I.A. 1-15-54	A.S.H.D. O.E.		
30	M.W. 65 WM 182064 4-23-54	+	N	S.T.	O.E. A.S.H.D.	5-24-54	a) Acute Thrombosis of Right Coronary Artery; b) Pulmonary Emphysema; c) Right Ventricular and Auricular Hypertrophy and Dilatation; d) Arteriosclerosis of all Coronary Arteries; e) Myocardial Fibrosis.
31	F.H. 65 WF 178185 12-29-53	+	+	S.T.	O.E. A.S.H.D.		
32	W.F. 62 WM 200472 7-20-55	+	++	D.P.T.	O.E. A.S.H.D.	8-6-55	a) Pulmonary Emphysema; b) Coronary Arteriosclerosis; c) Diffuse Myocardial Fibrosis; d) Pulmonary Edema.
33	C.D. 53 CM 177725 12-15-53	N	++	S.T. D.P.T.	O.E. H.C.V.D.	1-3-54	a) Silicosis; b) Emphysema; c) Right Ventricular and Auricular Hypertrophy and Dilatation.
34	J.H. 73 WM 59141 1-31-56	N	N	N	O.E. A.S.H.D.	1-24-57	a) Thrombosis, Recanalized, Right Circumflex Coronary Artery; b) Infarction, Old, Posterior Interventricular Septum; c) Cardiac Hypertrophy, Generalized; d) Pulmonary Edema; e) Pulmonary Emphysema.

CHART 4 (*Continued*)

	Patient Age, Color, Sex, Hist. #, Admission Date	Heart Size	Heart Size by X-ray	E.C.G.	Diagnosis	Date of Death	Autopsy Finding
35	R.T. 40 WM 215676 7-9-56	+++	N	S.T.	O.E. H.C.V.D.		
36	C.H. 55 WF 222817 12-29-56	+	+	—	O.E. H.C.V.D.		
37	E.W. 62 CM 228107 5-7-57	+	+	—	O.E. H.C.V.D.	5-11-57	a) Pulmonary Emphysema; b) Concentric Hypertrophy, Left Ventricle; c) Dilatation of Right Auricle and Ventricle; d) Acute Pulmonary Hemorrhage Secondary to Bleeding from Tracheotomy.

veins were distended, and there was severe ankle edema. The heart was enlarged to the anterior axillary line. The rhythm was regular. There was a harsh systolic murmur over the entire precordium, loudest at the base and transmitted into the neck vessels. There were medium moist rales at the lung bases posteriorly.

The liver was felt six centimeters below the right coastal margin. There was shifting dullness in the abdomen with a fluid wave. There was leg edema to the hips.

The serological test for syphilis was negative. The routine blood and urine studies were normal. The electrocardiogram was compatible with an old posterior myocardial infarction although no history suggestive of this condition could be obtained. The chest roentgenogram showed a boot-shaped heart with prominence of the pulmonary markings.

The patient was placed on a low salt diet, was fully digitalized and given intermittent thiomerin. On this regimen he lost weight rapidly (Chart 1) and was able to leave the hospital on December 2, 1953.

Comment: This 64 year old man had hypertensive and arteriosclerotic heart disease with possible aortic stenosis and was in severe left and right-sided heart failure when he entered the hospital. Chart 5 shows the return of ventilation, pH and carbon dioxide pressure toward normal with loss of the edema. The effect of the thiomerin given on the first day is impossible to assess because he was concomitantly put on bed rest, a low salt diet and digitalis.

It is of interest, however, that the diuresis during the first two days was not as extensive as those obtained following thiomerin on November 14 and 20 when the pH was normal.

Case 2: C.B. (B.C.H. #175430) a 55 year old negro came to the Baltimore City Hospitals on October 6, 1953 complaining of shortness of breath and a cough of three weeks' duration.

CHART 2

Physical Findings and Laboratory Data in Patients Who Had Basilar Pulmonary Rales at the Time of Study (Group I)

	B.P.	P.	R.	Orthopnea	Basilar Rales	Liver Enlargement (cm.)	Ankle Edema	Digitalis	Mercurial Diuretic	Weight	Miscellaneous	Study Date	V.C. x 100 / Pred. V. C.	M.B.C. x 100 / Pred. M.B.C.	Sat. (%)	P_{O_2} (mm. Hg.)	P_{CO_2} (mm. Hg.)	C_{CO_2} (mEq./L.)	pH	B.B. (mEq./L.)	V_E/m^2 B.S.A.	V_{O_2}/m^2 B.S.A.	D.S. x 100 / T.V.	Ht.
Normal Value*													100	100	96+	90+	40	22.00	7.40	50	3.2	129	30	42
1	170/110	100	30	+	+	1	+++	S	+	208		11-5-53			(93)	80	36	18.00	7.36	43	8.30	182	55	40
		96	26	+	+			C		204		11-6-53			(96)	91	29	19.10	7.50	46				37
		78	28	+	0		+++	M		193		11-9-53			(94)	69	30	20.09	7.50	48	8.69	198	43	39
		88	24	0	0					190		11-11-53			(93)	64	31	20.61	7.50	47				37
		94	30	0	0		++			188		11-13-53			(93)	73	33	20.29	7.41	45				36
		80	20	0	0				+	176		11-16-53			(95)	81	36	20.68	7.41	45	6.37	156	47	35
		84	22	0	0					172		11-18-53			(95)	74	31	19.68	7.48	47				38
		80	20	0	0		+		+	172		11-20-53			(94)	75	36	19.19	7.41	46	6.53	156	44	39
		78	18	0	0		0			162	NH_4CL	11-24-53			(93)	79	38	18.82	7.34	43	6.67	176	47	41
		86	20	0	0					160		11-27-53			(95)	86	39	20.62	7.37	45	6.91	175	44	39
	150/98	76		0	0					160		11-30-53			(95)	85	42	19.44	7.37	44	6.22	164	52	
					0					162		9-14-54	115	56										
2	170/108	100	38	+	+	6	++	M				10-7-53			(97)	79	24	20.25	7.62	52				41
		78	24	+	+				+	178		10-8-53			(97)	103	22	18.44	7.62	50	8.77	168	26	40
		86	24	0	+		0			166	NH_4CL	10-14-53			(99)	87	38	15.54	7.28	40	7.26	152	36	50
		94	20	0	0	0				161	NH_4CL	10-19-53			(97)	105	32	15.02	7.36	43	6.33	126	48	52
3	120/74	100	28	+	+	2	0	M	0			4-13-54			(84)	46	28	17.50	7.47	46				44
		88	20	0	0	2						4-19-54			(96)	94	36	20.09	7.42	46				45
	130/70	82	20	+	+	0					L.E.	10-17-54			(96)	70	22	18.25	7.62	50				43
4	150/30	120	36	+	+	8	+++	M	+			2-16-55			(97)	95	32	22.12	7.52	52				40
5	145/105	90	26	+	+	3	0		0			5-5-54			(94)	76	32	18.75	7.46	49				47
	140/115	120	36	0	+		0	S			Morphine	5-6-54			(94)	79	40	20.89	7.40	48				46
	116/78	90		0	0	0		M				5-14-54			(92)	71	41	21.29	7.39	48				44
6	220/130	150	35	+	+	5	++	S	0			2-16-54			(79)	41	32	19.95	7.46	48				42

* Normal Values. Using the nomogram of Singer and Hastings (14), P_{CO_2}, C_{CO_2} and Hematocrit are determined directly. pH and Buffer Base are estimated from the nomogram. The normal values for P_{CO_2} are 36 to 45 mm. Hg.; for C_{CO_2} 22.00 ± 2.00 mEq./L; for derived pH 7.36 to 7.46 and for Buffer Base 45 to 53 mEq./L.

He had been well until 1947 when he developed cardiac failure and was treated at another hospital with a low salt diet, digitalis, and periodic mercurial diuretics.

Approximately one month prior to admission the shortness of breath became worse, there was orthopnea and paroxysmal nocturnal dyspnea of increasing severity.

Examination on admission showed a temperature of 100.6° F., a pulse rate of 100, respirations of 30 and a blood pressure of 160/108. The patient was orthopneic and dyspneic. The neck veins were distended, and there was moderate ankle edema. The heart was enlarged to the left. The rhythm was regular. There were no murmurs. There were medium moist rales at both bases posteriorly. The liver edge was felt six centimeters below the right costal margin.

The serological test for syphilis was positive with a titer of 16 units. Routine blood and urine studies were not remarkable. The electrocardiogram was compatible with left ventricular hypertrophy. The chest roentgenogram revealed an enlarged boot-shaped heart with moderate pulmonary congestion.

The patient was placed on a low salt diet, digitalis 0.1 gm. daily, ammonium chloride 0.8 gm. daily four days each week, followed by intramuscular thiomerin. On this regimen, he improved rapidly, lost 17 pounds and was discharged on the fifteenth hospital day. He received penicillin for syphilis.

Comment: This 55 year old negro, with hypertensive cardiovascular disease and heart failure of six years' duration responded well to a low salt diet and mercurials. The studies (Chart 5) show that he was in severe respiratory alkalosis on admission. The pH, carbon dioxide tension and ventilation returned toward normal with treatment. On discharge, however, when he was asymptomatic, his ventilation was still twice the normal value.

Group II: This group differs from Group I in that there was no evidence of pulmonary congestion. No rales were present at the lung bases at the time of study. A few had orthopnea and some had ankle edema. In general, respiratory alkalosis was less frequent than in patients who had rales at the lung bases, and minute ventilation was not as great.

The patient listed as Case 12 had a severe posterior myocardial infarction, but had no signs or symptoms of heart failure. On March 15, 1954 there was a cerebral embolus, and the patient became comatose. A study done on this day showed a marked respiratory alkalosis caused by the hyperventilation associated with a cerebrovascular accident. The respiratory acidosis in Case 19 may have been associated with pregnancy. The history of a patient in this group follows.

Case 8: J.S. (B.C.H. #178947) a 50 year old colored woman was admitted to the Baltimore City Hospitals on January 20, 1954 complaining of ankle swelling and shortness of breath of three weeks' duration. The patient had rheumatic fever as a child.

The present illness started three weeks prior to admission with ankle edema. Swelling of the abdomen followed, and there was finally dyspnea and orthopnea.

On examination the patient had a temperature of 98.8° F., a pulse rate of 128, respirations of 28, and a blood pressure of 164/90. She was extremely orthopneic. There was edema of the right arm, the legs, and the lower abdominal wall. There were signs of a pleural effusion on the right. The heart was tremendously enlarged, the point of maximal impulse being in the

midaxillary line. The rhythm was regular. The second aortic and second pulmonic sounds were of equal amplitude. There was a grade II systolic murmur at the base and at the apex. There was a soft short diastolic murmur in the pulmonic area.

Because of edema of the abdominal wall, it was impossible to be certain about the size of the liver or the presence of fluid.

Blood and urine studies were not remarkable. The serological test for syphilis was positive. The electrocardiogram showed non-specific ST segment and T wave changes.

The patient was thought to be suffering from rheumatic heart disease with mitral insufficiency, aortic stenosis and possibly aortic insufficiency.

She responded well to bed rest, a low salt diet and digitalis. The weight fell from 214 to 147 pounds.

Comments: The studies (Chart 6) show that there was moderate respiratory alkalosis and a low arterial blood oxygen tension on January 21. Ammonium chloride given on January 26 brought the pH back to normal. On January 29, pH and oxygen tension had returned to normal. A gram of Diamox on February 2 and 3 caused a moderate metabolic acidosis without any change in the oxygen tension. On February 11, the pH and oxygen tension were normal.

Group III: Studies performed on three patients in severe pulmonary edema (terminal in two) showed an unusual pattern. The P_{CO_2} was normal or high; the C_{CO_2} was relatively low; in two patients the C_{CO_2} was below the normal limit. The changes resulted in a low pH. One patient (Case 22) survived the attack of pulmonary edema. Three studies performed while the patient remained in the hospital demonstrated a fall in the P_{CO_2} with return of the pH to normal. A study performed 19 months after the episode of pulmonary edema was normal except for decreased P_{O_2}. All patients demonstrated reduced oxyhemoglobin saturation. Case 24 had a history of asthma of several year's duration. Yet on physical examination and at autopsy there was no evidence of pulmonary emphysema.

Group IV: All patients in this group had clinical evidence of pulmonary emphysema. In three (Cases 25, 26 and 27), this evidence was only an increase in anterior-posterior chest diameter, low diaphragms and a hyperresonant percussion note. These three patients had normal or high P_{CO_2} at the time of study, but the low or relatively low C_{CO_2} suggests that they had previously been hyperventilating and had recently started ventilating normally. These patients had low pH.

The remainder of the patients in Group IV had elevated C_{CO_2} suggesting prolonged alveolar underventilation.

It is impossible to be certain of the significance of basilar rales in these patients and to differentiate the part played in the clinical situation between left ventricular failure and chronic or acute lung disease. As might be expected in such a complex clinical situation, the acid-base balance was variable. A high C_{CO_2} and P_{CO_2} was associated with the more severe degrees of obstructive

CHART 6

Physical Findings and Laboratory Data in Patients with Compensated Cardiac Failure (Group II)

	B.P.	P.	R.	Orthopnea	Basilar Rales	Liver Enlargement (cm.)	Ankle Edema	Digitalis	Mercurial Diuretic	Weight	Miscellaneous	Study Date	V.C. x 100 / Pred. V.C.	M.B.C. x 100 / Pred. M.B.C.	Sat. (%)	P_{O_2} (mm. Hg.)	P_{CO_2} (mm. Hg.)	C_{CO_2} (mEq./L.)	pH	B.B. (mEq./L.)	V_E/m^2 B.S.A.	V_{O_2}/m^2 B.S.A.	D.S. x 100 / T.V.	Ht.
Normal Values													100	100	96 +	90+	40	22.00	7.40	50	3.2	129	30	42
7	140/80	60	20	+	0	3	+	M		189		10-29-53			(92)	69	42	20.94	7.36	46	6.28	167	56	39
		78	22	+	0		+			190		1-8-54			(95)	84	38	21.49	7.43	49	5.93	153	60	45
		80	20	0	0		+			190	NH_4Cl	1-12-54			(94)	78	38	18.60	7.38	44	6.62	147	48	43
		76	24	0	0		+					1-14-54									5.86	149	42	
		76	20	0	0		+					1-15-54			(94)	76	38	22.50	7.45	51				43
	130/80	78	22	0	0		+				Diamox	1-19-54			(94)	85	41	19.47	7.34	44	6.55	139	52	43
		80	20	0	0	2	+			185	NH_4Cl	9-14-54												
		74	24	0	0		+			187	NH_4Cl	9-21-54	63	70	(95)	84	36	18.81	7.40	45				45
8	135/76	76	28	+	0	10	+++	S	+	214		1-21-54			(95)	77	34	21.66	7.48	50				42
		80	20	+	0	10	++	C		170		1-25-54			(95)	73	40	24.34	7.48	53				44
		84	22	0	0	5	++	M		163	NH_4Cl	1-27-54			(95)	84	40	21.29	7.41	48				45
		78	20	0	0	2	+			159		1-29-54			(96)	93	38	21.26	7.42	49				44
		80	20	0	0	0	0			152		2-1-54			(96)	90	42	23.64	7.42	50				41
		80	20	0	0	0	0			145	Diamox	2-3-54			(95)	90	45	20.06	7.32	44				45
		80	22	0	0	0	0			152		2-11-54			(96)	83	44	22.19	7.37	47				41
9	180/120	80	20	0	0	0	0				Morphine	2-25-54			(82)	48	39	21.11	7.40	47				42
	145/105	104	24	0	0	0	0					2-26-54			(83)	50	42	22.16	7.40	49				43
	130/100	92	18	0	0	0	0	C	0			3-1-54			(94)	81	42	22.57	7.41	48				39
	94/50	80	18	0	0	0	0					3-15-54			(95)	87	25	22.23	7.60	50				35
	130/70	70	18	0	0	0	0					3-17-54			(95)	83	31	20.69	7.48	46				31
10	115/90	100	24	+	0		+++	M	+	162	P.B.	5-27-54			(93)	78	35	17.26	7.36	42				39
		88	20	0	0		+		+	144		6-2-54			(92)	77	37	20.03	7.32	42				35
		88	22	0	0		0			131	Diamox	6-9-54			(93)	80	35	18.11	7.37	41				34

		88	22	0	0		0			131	Diamox	6–9–54			(93)	80	35	18.11	7.37	41				34
11	95/72	92	24	0	0	2	0					4–1–54			(92)	70	35	19.78	7.43	45				45
	94/60	88	24	0	0		0		0			4–5–54			(94)	74	33	21.88	7.50	50				41
	80/60	108	28	0	0		0	C				4–7–54			(94)	80	37	20.00	7.40	45				43
12	96/68	104	24	0	0	0	0					7–13–54			(90)	63	35	20.40	7.42	45				36
	80/60	90	20	0	0	0	0		0			7–21–54			(93)	70	32	19.24	7.46	47				36
	104/68	80	18	0	0	0	0	C				9–16–54	74	38	(98)	103	28	19.27	7.52	50	6.32	139	23	42
13	270/150	100	20	+	0	1	0	S				4–12–54			(96)	94	35	19.83	7.41	45				44
	140/80	84	20	+	0		0		0			4–19–54			(96)	88	39	20.69	7.39	46				39
	120/80	60	20	0	0		0					4–27–54			(96)	90	36	22.28	7.47	50				39
14	200/40	80	20	+	0	0	++	M	+		Ascites	9–17–54			(96)	91	33	18.91	7.44	45				38
15	110/70	112	48	+	0	0	+++	M	0			7–12–55			(90)	62	34	19.69	7.38	44	6.40	130	49	34
16	130/80	78	20	0	0	5	0	M	0			5–7–55			(94)	72	30	20.96	7.52	50				40
17	130/85	64	18	0	0	3	0	S	0			2–16–54	96	102	(97)	94	26	20.36	7.56	48				30
18	130/80	88	22	0	0	0	0	M	0			2–2–54			(95)	82	40	24.14	7.44	51	4.04	160	37	38
19	100/70	88	20	+	0	0	0	0	0			11–12–54	51	36	(98)	110	31	19.62	7.45	46	4.35	132	36	39
		84	18	0	0	0	0					11–18–54	78	54										
20	110/80	84	18	0	0	0	0	0	0		6 mo.	9–28–54	78	46										
	110/80	80	20	0	0	0		0	0	96	Pregnant	7–22–55	58	52	(98)	101	21	18.33	7.58	44	5.64	154	14	25
21	98/50	84	20	0	0	0	0	M				9–10–54	89	50	(95)	85	31	18.00	7.42	43	7.12	173	52	33

CHART 7

Physical Findings and Laboratory Data in Patients With Severe Pulmonary Edema (Group III)

	B.P.	P.	R.	Orthopnea	Basilar Rales	Liver Enlargement (cm.)	Ankle Edema	Digitalis	Mercurial Diuretic	Miscellaneous	Study Date	Sat. (%)	P_{O_2} (mm. Hg.)	P_{CO_2} (mm. Hg.)	CO_2 (mEq./L.)	pH	B.B. (mEq./L.)	Ht.
Normal Values												96+	90+	40	22.00	7.40	50	42
22	195/120	160	24	+	+	1	0	C	0	P.E.	10–13–53	(86)	56	39	18.04	7.34	44	49
		90	26	0	0	1	0				10–20–53	(96)	89	33	17.68	7.39	43	37
		78	20	0	0	1	0				10–29–53	(94)	78	32	18.26	7.43	45	40
	220/120	84	24	0	0	1	+				5–4–55	(89)	59	41	23.99	7.43	50	37
23	110/60	140	35	+	+	6	+	0	0	P.E.	3–18–54	(91)	72	47	21.38	7.30	44	35
24	158/140		40	+	+	0	0	0	0	P.E.	10–7–54	(52)	37	62	16.75	7.10	36	61

emphysema, but the ratio between these two parameters, as shown in the pH, was quite variable. Where the pH was very high in the presence of a high C_{CO_2}, there must have been recent relative alveolar hyperventilation possibly induced by recent left ventricular failure. Where the C_{CO_2} was elevated and the pH was low, there must have been recent relative alveolar hypoventilation associated with bronchial obstruction, possibly caused by bronchospasm, bronchiolar edema or bronchiolar infection. To differentiate cardiac from pulmonary mechanisms affecting ventilation in this situation seems impossible.

DISCUSSION

Changes in the acid base balance and the oxygen tension of arterial blood are closely related to the adequacy of alveolar ventilation, to the activity of the kidney in response to changes in P_{CO_2} and pH, and to the distribution of air to the various parts of the lungs.

I. Alveolar Ventilation, P_{CO_2} and Acid-Base Balance in Heart Failure

Although a large number of variables affect the alveolar carbon dioxide pressure slightly (16), alveolar hyperventilation is the immediate cause of the respiratory alkalosis of heart failure.

Since the arterial and alveolar carbon dioxide tension are equal, a rise in alveolar ventilation results in a lowering in arterial P_{CO_2}. The pH therefore, tends to rise and produces a respiratory alkalosis. The situation in respiratory acidosis is the exact opposite of the present situation and has been discussed in detail previously (3).

Charts 9 and 10 record the physiological patterns present in the different clinical situations.

Patients in Cardiac Failure with Basilar Rales

In this clinical situation there is alveolar hyperventilation (Chart 10). Respiratory alkalosis is the rule (81 per cent of the studies) and it is usually uncompensated. The uncompensated nature of the alkalosis emphasizes the instability of the acid-base situation in patients in inadequately compensated heart failure. Alveolar ventilation may change rapidly depending on changing states of exertion, position in bed, response to therapy, and degree of apprehension. pH depends on the ratio of bicarbonate to carbonic acid. Since bicarbonate changes relatively slowly compared to carbonic acid, and since carbonic acid levels are instantaneously changed by changes in alveolar ventilation, one might expect wide variations in the pH in heart failure.

Patients in Cardiac Failure Without Basilar Rales

In this situation the pattern is similar to that where cardiac failure is more marked, but more studies showed normal values. A little more than half the

CHART 8

Physical Findings and Laboratory Data in Patients Who Have Evidence of Pulmonary Emphysema and Heart Disease (Group IV)

	B.P.	P.	R.	Orthopnea	Basilar Rales	Liver Enlargement (cm.)	Ankle Edema	Digitalis	Mercurial Diuretic	Weight	Miscellaneous	Study Date	V.C. x 100 / Pred. V.C.	M.B.C. x 100 / Pred. M.B.C.	Sat. (%)	P_{O_2} (mm. Hg.)	P_{CO_2} (mm. Hg.)	C_{CO_2} (mEq./L.)	pH	B.B. (mEq./L.)	Ht.
Normal Value													100	100	96+	90+	40	22.00	7.40	50	42
25	135/80	88	20	0	0	0	0	0	0		Morphine	6-1-54			(87)	89	42	17.64	7.27	40	43
	130/100	96	22	0	0	0	0					6-3-54			(95)	95	39	19.52	7.34	43	44
		88	20	0	0	0	0					6-10-54			(96)	96	42	19.92	7.36	44	42
26	110/70	120	46	0	+	3	+	0	0			7-12-56			(82)	54	42	16.93	7.28	41	49
		98	24	0	0	0	0	C				7-17-56			(88)	60	32	20.33	7.51	52	50
27	200/60	80	16	0	0	3	0	M	0			3-22-54			(71)	46	57	21.53	7.21	40	33
28	120/65	88	24	0	0	0	0	M				10-22-53			(94)	67	33	25.11	7.58	55	35
		86	20	0	0	0	0					10-23-53			(84)	56	46	25.47	7.32	48	38
		82	20	0	0	0	0		0			10-26-53			(93)	66	37	25.37	7.50	52	35
		80	20	0	0	0	0					10-28-53			(91)	66	42	24.64	7.41	48	35
29	140/90	90	20	+	+	0	+	C	+			1-18-54			(85)	48	40	29.48	7.56	60	32
	145/90	80	18	0	0	0	+					1-20-54			(90)	58	40	25.76	7.46	51	34
30	140/100	126	32	+	+	7	+++	M	0		Ascites	4-29-54			(69)	40	59	26.05	7.33	51	48
31	140/110	90	24	0	0	0	0	M	0		NH_4CL	1-7-54			(93)	52	58	27.63	7.38	53	43
		88	20	0	0	0	0					1-8-54			(84)	57	51	23.83	7.34	48	43

32	110/80	120	38	+	+	3	+	M				7-21-55			(81)	46	51	27.72	7.41	54	44
		86	20	+	+	5	0		0			7-25-55			(85)	49	48	28.38	7.45	56	45
		106	26	+	+	5	0					7-28-55			(94)	72	38	27.56	7.56	59	48
33	198/160	80	25	+	+	2	+++	M	0			12-16-53			(68)	36	57	27.41	7.40	57	56
34	140/78	80	26	0	0	0	0	M	0			9-27-56	46	21	(93)	73	46	25.73	7.43	53	44
35	170/110	90	24	+	+	7	++	M	0			7-19-56	66	49	(97)	89	47	25.18	7.40	51	43
	165/95	64	18	0	0	0	0					10-17-56			(94)	77	45	25.39	7.42	52	40
36	170/70	88	20	+	+	3	++	M	0			1-2-57	68	40	(86)	60	67	29.23	7.31	54	51
37	170/120	118	36	0	+	6	0	M	0			5-10-57			(82)	43	61	38.69	7.50	70	54

CHART 9

Range, Mean Values and Standard Deviations of Arterial Blood Values in Different Groups

		With Rales	Without Rales	Group 4
P_{O_2}	Number of Observations	11	44	24
	Range	41–103	50–110	36–96
	Mean	77	80	62
	S.D.	18	12	17
P_{CO_2}	Range	22–40	21–44	33–67
	Mean	30	35	47
	S.D.	6	5	8
C_{CO_2}	Range	15.54–22.12	17.26–24.34	17.64–38.69
	Mean	18.99	20.64	25.16
	S.D.	2	1.7	4
pH	Range	7.28–7.62	7.32–7.60	7.21–7.58
	Mean	7.48	7.44	7.40
	S.D.	.35	.07	.28
B.B.	Range	40–52	42–53	40–70
	Mean	48	47	51
	S.D.	3.6	3.5	7

CHART 10

Ventilatory, Acid-base and P_{O_2} Findings in Various Clinical Conditions

Type of Patient	Studies										
	Ventilation			Respiratory Acidosis		Respiratory Alkalosis		Metabolic		Low P_{O_2}	Total Studies
	Normal	Increased	Decreased	Uncompensated	Compensated	Uncompensated	Compensated	Acidosis	Alkalosis		
Heart Failure with Basilar Rales	3	8				6	2	2		8	11
Compensated Heart Failure Without Basilar Rales	28	25		5		11	12	14		32	53
Cardiac Pulmonary Edema	1		2	3				3		3	3
Heart Disease and Emphysema	10	2	12	8	6	6		5	8	22	24

patients had normal alveolar ventilation, as judged by the P_{CO_2}. Alkalosis was again a prominent feature (62 per cent of studies) but the proportion of studies showing compensated respiratory alkalosis was increased.

Eleven per cent of the studies showed an uncompensated respiratory acidosis, suggesting that alveolar ventilation was decreasing in comparison to its

previous state, thus shifting the bicarbonate-carbonic acid ration toward a lower pH. This finding is what might be expected in rapid cardiac compensation.

Patients with Severe Cardiac Pulmonary Edema

There were several surprising findings in the studies performed during severe pulmonary edema. One might expect to find a marked uncompensated respiratory alkalosis with marked hyperventilation in patients with pulmonary edema. In Cases 22 and 24 the C_{CO_2} was indeed slightly low, suggesting that there had been a respiratory alkalosis in the past. In all three studies however, the P_{CO_2} was high in relation to C_{CO_2}, resulting in a low pH or uncompensated respiratory acidosis. In Cases 23 and 24, P_{CO_2} was elevated. These findings suggest that there had been hyperventilation with respiratory alkalosis in the past, but with the development of severe overwhelming pulmonary edema, alveolar ventilation was decreased. Case 24 had a history of asthma, and it is quite possible that cardiac pulmonary edema may precipitate bronchospasm in patients with asthma. The recent demonstration (17) of increased airway resistance during expiration in patients with pulmonary edema as well as the clinical findings of wheezing and the dramatic response to aminophylline in some patients suggests that bronchospasm is commonly associated with cardiac pulmonary edema.

Patients with Cardiac Disease and Pulmonary Emphysema

In this group the findings were much more variable, as one might expect in a situation where cardiac failure is tending to produce hyperventilation with respiratory alkalosis, and obstructive emphysema is tending to produce alveolar underventilation with respiratory acidosis. From a clinical standpoint, it was difficult to assess what part the heart and lungs were individually playing in the production of symptoms. It is evident from the studies that in Cases 25, 26 and 27 the emphysema did not play as significant a physiological role as it did in Cases 28 to 37. In the latter cases, however, it is difficult to assess what part the heart played.

Therapeutic Considerations in Relation to Alveolar Ventilation in Heart Failure

Respiratory alkalosis may be combatted either by reducing ventilation or by producing a metabolic acidosis. Ventilation may be reduced by the use of morphine, and, in some cases, by inhalation of a high oxygen mixture. This type of therapy is most appropriate in acutely dyspneic patients in severe heart failure. Ammonium chloride and Diamox are effective in promoting a metabolic acidosis. The use of oxygen, morphine and acidifying agents is theoretically sound for the correction of the acid-base balance and is also

empirically effective in the promotion of diuresis and relief of anxiety and dyspnea.

These measures (use of oxygen and morphine) have also proved to be helpful empirically in the treatment of severe pulmonary edema (Group III). If the findings present in all three patients in Group III are representative, one might expect morphine to further reduce ventilation and increase the respiratory acidosis. We have no assurance that these studies are representative, however, and suspect that they may be the findings in terminal and generally irreversible pulmonary edema. Studies before and after the use of morphine and oxygen are clearly indicated in severe pulmonary edema to elucidate this problem. It may be that bronchospasm is relieved by morphine and oxygen, so that the reduction in ventilation observed clinically is not a direct effect of the morphine depressing the respiratory center, but of an improvement in airway obstruction with an actual increase in alveolar ventilation. The fact that aminophylline, (which acts as a bronchodilator) may decrease ventilation, indicates that ventilation is not depressed by central mechanisms but is improved because obstruction is relieved.

In emphysema associated with heart failure, the situation is different since chronic hypoventilation is present. Where obstructive emphysema is suspected in a patient with heart disease, morphine and oxygen are to be avoided at all costs. The depression of respiration caused by these agents may be fatal. In a situation where it is not clear whether a patient has emphysema with an acute pneumonia or bronchitis or whether there is left ventricular failure, it is best to concentrate on the use of bronchodilators, particularly aminophylline, until a carbon dioxide content or carbon dioxide tension of arterial blood can be obtained to elucidate whether respiratory acidosis is present.

II. Distribution of Air Within the Lungs and P_{o_2}

A large number of variables affect the arterial blood oxygen tension (18). The homogeneity of the distribution of inspired air is the main factor influencing alveolar oxygen tension (19). If the inspired air goes to all alveoli in a uniform manner, all blood passing through the lungs will be fully oxygenated and arterial P_{o_2} will be normal. If some areas of the lungs are not well ventilated with each breath, mixed venous blood will pass through the lungs without being fully ventilated, and arterial P_{o_2} will be low.

The patients studied in this series tended to have low or normal arterial blood oxygen tensions. There was some improvement in the oxygen tension as compensation occurred but there was never a rise to high normal levels which one might expect with comparable hyperventilation in normal subjects. This finding suggests that inhomogeneity of ventilation existed in the lungs of these patients despite full clinical cardiac compensation. We have described this finding in patients with mitral stenosis (2) and have suggested that the

unequal ventilation of the lungs may be secondary to subclinical edema of the bronchi.

Charts 9 and 10 summarize the findings in the different groups. The mean oxygen tension was lowest when emphysema was associated with heart disease (62 mm. Hg.), highest in compensated heart failure (80 mm. Hg.) and intermediate when pulmonary rales were present. The P_{O_2} was low in all patients in severe pulmonary edema (Group III) in 92 per cent of Group IV, in 73 per cent of those with basilar pulmonary rales and in 73 per cent of those in cardiac compensation.

Therapeutic Considerations in Relation to P_{O_2}

The inhalation of high oxygen mixtures in all these situations will, of course, elevate the arterial blood saturation. It has been suggested above that full saturation may promote underventilation when chronic carbon dioxide retention is present, but in all other situations it is probably beneficial.

Taking all the studies into consideration, the rapid changes occurring in the acid-base balance as patients go into and recover from cardiac failure are apparent. Alveolar ventilation is a quickly changing parameter being followed sluggishly by changes in carbon dioxide content. Since pH is the resultant of the ratio between bicarbonate and carbonic acid, it will be changed immediately by variations in ventilation and more slowly compensated by renal mechanisms. Thus, sudden left ventricular failure, undue exertion, anxiety or other mechanisms that might produce hyperventilation throw the patient into uncompensated respiratory alkalosis. After several hours, provided ventilation remains steadily increased, compensation of the alkalosis will result. If the ventilation is now suddenly decreased relative to its recent volume, a respiratory acidosis will occur with a low carbon dioxide content.

SUMMARY

Studies of the acid-base balance and oxygen tension of arterial blood have been performed in a series of patients in various degrees of heart failure. Some patients have been followed over long periods of time with serial studies. Patients in various degrees of left ventricular failure generally were found to have respiratory alkalosis and low oxygen tension. Patients in severe pulmonary edema had variable findings, one of the surprising ones being severe acute respiratory acidosis. Patients with heart disease and pulmonary emphysema tended to have respiratory acidosis.

The physiological causes of these abnormalities and therapeutic implications are discussed.

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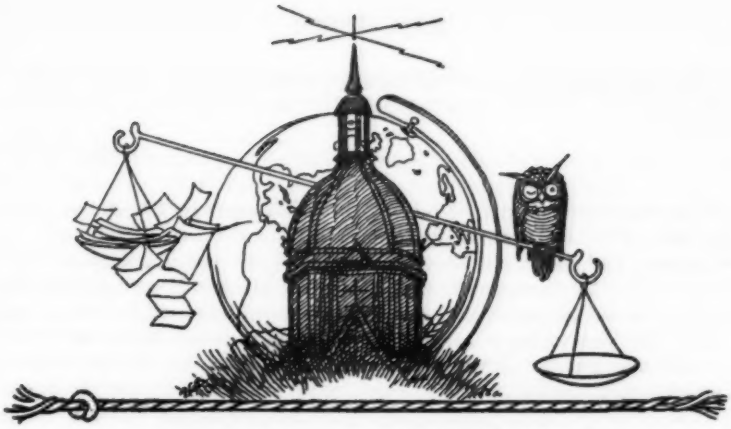

BOOK REVIEWS

War Blinded Veterans in a Postwar Setting. A Social Work Followup of Rehabilitation Measures for Blinded Veterans with Service-Connected Disabilities Between December 7, 1941 and March 31, 1953. 260 pp., $1.50. U. S. Government Printing Office, Washington, D. C.

This little book, consisting of 60 pages of manuscript and 200 pages of tables, appendices and indexes, is intended as a statistical summary of the work done by the Veterans Administration in the rehabilitation of the approximately 2,000 blind or partially blind veterans of the 2nd World War and the Korean War. Under the supervision of a number of highly skilled social workers in Veterans' Centers, all the war-blinded veterans were asked to answer more than a hundred questions and their answers tabulated. The result is of considerable interest to all those dealing with young blind people.

Of the 2,000, 1,000 were blinded by accidents, most of them on active duty; the remainder contracted diseases which left them blind. Most often if blindness resulted from wounds received in action, there were other serious physical disabilities. Still, 60 per cent of the partially blind and 40 per cent of the totally blind are able to support themselves working. Many own their own homes and have families. Implicit in this compendium of statistical material is the tireless work of the division of Vocational Rehabilitation and Education. It is a continuing job well done. The book is well indexed.

STEWART M. WOLFF

Action of Radiation on Tissues. An Introduction to Radiotherapy. By A. LACASSAGNE AND G. GRICOUROFF. Translated by Clarence C. Lushbaugh and Gretchen R. Riese. 199 pp., $6.25. Grune & Stratton, Inc., New York, N. Y.

This little book is designed for resident physicians and for those desiring a very fundamental concept of the action of radiation on tissues in preparing for work in radiotherapy or in radioisotopes. It is a good translation from the French. There are a few well chosen illustrations. There is an initial short chapter on the physical concepts of radiation action on the individual cells. Subsequently radiation action on the various body systems are treated. Many of the chapters are only a few pages long. The text material is distinct and accurate. All pertinent references are included at the end of each chapter. Finally the effects of total body radiation and the action of radiation on diseased tissues are discussed. There are good author and subject indexes.

This book is recommended to medical students and physicians who require a concise, not too technical, review of present knowledge of how the organism and the mammalian respond to radiation.

DANIEL TORRANCE

Care of the Premature Infant. By EVELYN C. LUNDEEN AND RALPH H. KUNSTADTER. 367 pp., $8.00. J. B. Lippincott Co., Philadelphia, Pa.

This volume by Lundeen and Kunstadter represents, in essence, a third edition of the well known book "The Premature Infant" by Hess and Lundeen (Second Edition 1949). It represents an up to date, superficially complete treatise on all aspects of care of the premature infant. The presentation of material is orderly and the result highly readable. While many would differ with the authors as to some of the details of care, such as the insistence upon breast feeding, this book should be a useful guide to nurses in terms of: 1) the management of the premature infant himself; 2) his immediate environment during those difficult early weeks; and 3) home care and follow-up.

JANET B. HARDY

Regional Ileitis, 2nd revised edition. By BURRILL B. CROHN AND HARRY YARNIS. With Special Contributions by Richard H. Marshak and David A. Turner. 239. pp., $7.25. Grune & Stratton, New York, N. Y.

Dr. Crohn and Dr. Yarnis describe their experience with 676 cases of regional ileitis and related disorders seen at the Mount Sinai Hospital over the past 25 years. Three hundred and seventy-eight cases have been added to their series since Dr. Crohn wrote the first edition 8 years ago. In addition to an analysis of the clinical material seen at the Mount Sinai Hospital, the authors compare their experience with the reports of others. This combined experience with this difficult disease is recorded in an extensive bibliography.

The form and chapter headings conform in general to the first edition, but the emphasis has changed as to the role of expectant management and the timing of surgical intervention in the treatment of regional enteritis. In the first edition it is said that it is an open question whether any conservative or expectant form of medical treatment is warrantable in regional ileitis. Further observations have led the authors to add a new chapter which deals with the "Prognosis in Regional Ileitis Under Medical or Conservative Treatment." The course of 85 patients followed for one to twenty-five years is described in this chapter. Fifty-one have been classified as "well" and fifteen as improved.

The Mount Sinai Hospital group favors short circuiting type procedures over resection in the treatment of regional ileitis, but most of the differences they present are not statistically significant. In addition, it is difficult to determine which deaths in their series were operative.

The new edition has a chapter on "Radiographic Diagnosis of Regional Ileitis, Ileojejunitis and Ileolitis" by Richard H. Marshak, which comes before ileojejunitis and ileocolotis are described in the text. The new chapter on "Malabsorption in Ileitis" by Dr. David Turner is sandwiched between chapters on "Ileojejunitis" and "Prognosis and Treatment of Ileojejunitis."

This book offers a concise discussion of our understanding of regional ileitis and its related or more unusual forms, such as acute ileitis, ileojejunitis and ileocolitis. The extensive bibliography makes this little book a valuable reference for anyone dealing with the problems of regional enteritis.

THOMAS R. HENDRIX

The Epileptic Seizure. Its Functional Morphology and Diagnostic Significance. By COSIMO AJMONE-MARSAN AND BRUCE L. RALSTON. 251 pp., $6.00. Charles C Thomas, Springfield, Ill.

This timely monograph on the epileptic seizure describes in a precise crisp manner the totality of clinical and electroencephalographic events during the activation of the epileptic process by metrazol. The pertinent literature on this subject is critically reviewed. In the hands of the authors, the induction of full blown seizures by metrazol has not been hazardous. It must be remembered, however, that these men are highly skilled in the management of epilepsy and they have facilities to handle most any emergency complication of a fit. The evidence from their data clearly indicates that metrazol activation is an invaluable aid in the more accurate localization of epileptic foci.

Meticulous attention to detailed patterns of seizure development provides clinical guides to anatomical localization and probable sites of spread of the epileptic discharge. A scheme of seizure formulae is proposed which further aids in more accurate seizure localization in the brain.

The patient material is somewhat selected in that the National Institute of Neurological Diseases and Blindness is a referral center for patients refractory to the usual attempts at medical regulation, and many of these are considered suitable candidates for surgical excision of epileptogenic foci. This selection is evident in the preponderance of patients with temporal lobe seizures which is different from the average large general hospital experience in patients with epilepsy.

There is a wealth of tabulated statistical data on various types of seizures. The illustrations of facial and extremity postures with seizures of different anatomical origin are especially excellent. They emphasize the need and value of careful clinical observations of patients during an epileptic attack. The authors rightly point out that there is a much higher incidence of demonstrable brain pathology in patients with suprasylvian versus infrasylvian seizures.

It is a bit surprising to learn that angiography has not been a very useful adjunct in the diagnostic workup in this series of patients. On most neurosurgical services this is hardly what one would expect. Again, however, this would appear to be a matter of selection where contrary to the experience of these authors, most neurosurgical divisions deal with a higher percentage of patients with expanding intracranial lesions with epilepsy and demonstrable arteriographic abnormalities.

This monograph represents an important milestone in our better understanding of epilepsy, and anatomical localization of seizure discharges. There is good correlation with electroencephalographic findings. This is a must for the library of these physicians intimately concerned with the diagnosis and management of epilepsy.

CHARLES N. LUTTRELL

Surgery of Head and Neck Tumors. By HAYES MARTIN. 430 pp., $18.50. Paul B. Hoeber, New York, N. Y.

One of the foremost authorities in the field has drawn upon his vast experience to produce this text of the operative treatment of cancer of the head and neck. The author makes no attempt to review the literature or to arbitrate conflicting opinions but presents his personal views on the topic, an approach which would seem to have considerable merit. The subject matter includes not only the author's surgical approach but also his philosophical approach to the therapy of tumors of this region, an aspect which has proved difficult to many engaged in treatment of patients with such lesions.

About one-fourth of the volume is devoted to an exposition of the basic principles of cancer

surgery as applied to the head and neck and includes consideration of the preoperative diagnosis and treatment, the general principles of the operative procedures, postoperative care and complications, and the cosmetic and functional disabilities which follow radical surgery in this area. This discussion is comprehensive and thorough. No consideration is given, however, to the results obtained by surgical therapy in the various lesions. Neither is there any discussion of the pathology of tumors of this region, nor is the role of irradiation in the treatment of head and neck cancer discussed.

The remainder of the volume is composed of an atlas of the operative procedures utilized in this region. Almost 600 line drawings illustrate these procedures, each accompanied by a brief descriptive paragraph of the technique illustrated. The presentation is sufficiently complete without being cumbersome. Consideration is given not only to the excisional but also the reconstructive techniques employed.

The organization of material lends itself to easy use of the book as a reference text. The print is large and the paper of dull finish. The illustrative drawings are of good quality.

Although its scope is limited, this book should find a definite place in the literature of the field. It will be particularly valuable to those learning head and neck surgery but the personal approach to the subject by an acknowledged leader in this specialty should also be of considerable interest to those established in the field.

JAMES R. CANTRELL

BOOKS RECEIVED FOR REVIEW

Administrative Medicine. Transactions of the Fifth Conference Oct. 29, 30 and 31, 1956. Edited by GEORGE S. STEVENSON. 197 pp., $3.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

A Bibliography of Internal Medicine. Communicable Diseases. By ARTHUR L. BLOOMFIELD. 560 pp., $10.00. The University of Chicago Press, Chicago, Ill.

A Doctor Speaks His Mind. By ROGER I. LEE. 130 pp., $3.00. Little, Brown & Co., Boston, Mass.

Hormone Production in Endocrine Tumors, Vol. XII. With Cumulative Index to Volumes I–XII. Ciba Foundation Colloquia on Endocrinology. Edited by G. E. W. WOLSTENHOLME AND MAEVE O'CONNOR. 351 pp., $9.00. Little, Brown & Co., Boston, Mass.

Physiology of Prematurity. Transactions of the Second Conference March 25, 26 and 27, 1957. Edited by JONATHAN T. LANMAN. 160 pp., $3.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

Progress in Cardiovascular Diseases. Edited by CHARLES FRIEDBERG. Vol. 1, No. 1. 109 pp. Grune & Stratton, New York, N. Y.

Psychiatry for Nurses, 5th edition. By LOUIS J. KARNOSH AND DOROTHY MERENESS. 406 pp., $4.50. The C. V. Mosby Co., St. Louis, Mo.

Scientific Principles in Nursing, 3rd edition. By M. ESTHER MCCLAIN AND SHIRLEY HAWKE GRAGG. 535 pp., $4.50. The C. V. Mosby Co., St. Louis, Mo.

Surgery and Surgical Nursing, 3rd edition. By EDWARD S. STAFFORD AND DORIS DILLER. 469 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa.

AN EXPERIMENTAL STUDY OF THE NON-FIBRILLAR COMPONENTS IN FROG STRIATED MUSCLE[1]

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In the past few years numerous papers have appeared concerning the structure of the sarcoplasmic reticulum in striated muscle. The most extensive electron microscope studies of this cytological constituent in striated muscle are those of Bennett and Porter (4), Porter (7), Bennett (5), Edwards, Ruska, Souza Santos, and Vallejo-Friere (6), Porter and Palade (11), and Andersson (9). From these morphological studies of the sarcoplasmic reticulum two possible functions have been postulated. These are: 1). the internal transmission of action currents from the sarcolemma to the contractile apparatus and 2). the distribution of metabolites or some other metabolic role. Although no experimental evidence is presented by these authors the internal conduction hypothesis is the more strongly favored of the two suggested functional roles. This view has been further supported, but only by additional morphological studies by Moore and Ruska (10), Edwards, Ruska and de Harven (13, 14) and Ruska, Edwards and Caesar (15). Fawcett and Cecily (12) have pointed out, however, that in the turtle heart the sarcoplasmic reticulum is, at best, very poorly developed or absent and therefore the hypothesis of internal conduction could not be confirmed in this tissue. From morphological considerations neither the internal conduction hypothesis nor the metabolic distribution hypotheses can be disregarded but must be considered as extremely tentative until a wide variety of experimental evidence is available. Huxley (8) has provided evidence through the use of microelectrodes that striated muscle can be activated only in the region of the Z band. These observations have been interpreted by Ruska, *et al.*, (15) to indicate internal excitation via the sarcoplasmic reticulum.

The present study is a preliminary report based on the rather simple procedure of isolating frog sartorius muscles from their nervous and vascular supply, stimulating the muscle periodically over a period of 30, 45, and 60 minutes, and recording the muscular contractions. Isolated muscles which did not change appreciably in the amplitude of contraction during the experimental period were fixed carefully and studied by electron microscopy.

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

The author is indebted to Professor Bodian for the interest shown and encouragement given during the progress of this study. In addition, the technical assistance of Mrs. V. Prinn and Mrs. N. Taylor is gratefully acknowledged.

In addition, normal controls were studied. The results of this study indicate that in the experimental muscle cells the contractile apparatus and other components of the striated muscle cell, with the exception of the sarcoplasmic reticulum and mitochondria, remained unchanged. Studies of glycerol extracted striated muscle revealed the sarcoplasmic reticulum to be composed of two distinctly different components. Thus, the unitary concept of the sarcoplasmic reticulum in striated muscle and its proposed functional roles must be re-evaluated and reinvestigated. The discussion section will consider this in some detail.

MATERIALS AND METHODS

A total of six frogs were used in the isolation experiment in which sartorius muscles were tested for contractility for periods of 30, 45, and 60 minutes. Two frogs were used for each of the experimental periods with one muscle from each frog serving as a control for the experimental muscle. A total of six sartorius muscles served as experimental controls.

The sartorius muscles of frogs were removed at room temperature (27°C.) and connected to an isotonic muscle lever. The lever, equipped with an ink writer, recorded the muscle activity on a kymograph drum. The lever and ink writer corresponded to a direct loading on the muscle of about 10 grams. A stimulus (approx. 10 v.), producing a maximal response, was delivered through platinum electrodes to the origin of the muscle every few minutes for the experimental period (30, 45, and 60 minutes). Both single and multiple pulses (4/sec). were employed. The muscles were frequently bathed in frog Ringer solution to prevent drying. After the experimental period, the muscles were fixed by immersion in isotonic, buffered (pH 7.2–7.4), 1 per cent osmium tetroxide for 1 hour. Normal controls were treated after removal from the animal by the same fixative described above. Each muscle provided at least 20 specimens. After washing the tissue in frog Ringer for 1 hour and dehydration in graded alcohols, the tissue was embedded in a mixture of methyl and butyl methacrylate (1:9). Polymerization aided by the catalyst benzoyl peroxide was carried out at 40°C. Four additional control muscles were treated with phosphotungstic acid (0.1 per cent in graded alcohols) during the dehydration process to enhance tissue contrast and were then embedded as described above. Ultrathin sections were cut with Sjöstrand and Porter-Blum microtomes, mounted on formvar-coated copper grids, and studied in an RCA EMU 3c electron microscope.

In addition to the above procedures, four frog sartorius muscles were extracted in 50 per cent glycerol at 0°C. for 3 days. Following the extraction the muscles were fixed in 1 per cent osmium tetroxide and prepared for electron microscopy as described above.

RESULTS

The appearance of the sarcoplasmic reticulum in control sartorius muscles is consistent with earlier descriptions. The predominant longitudinal components appear tubular while the transverse components, which are less readily demonstrated, appear membranous in frog striated muscle. The transverse components are associated with the sarcolemma and myofibrils at the level of the Z and M bands, while the longitudinal components are found in the subsarcolemmal and interfibrillar spaces. Although the longitudinal and transverse

FIG. 1. Longitudinal section of frog sartorius muscle. Normal control. Elements of the longitudinal and transverse reticulum are indicated by white arrows. Mitochondria are designated by the white letter M while the characteristic muscle banding is indicated by black letters. The black arrow in the lower right hand corner indicates the sarcolemma. Contrast of specimen enhanced by phosphotungstic acid. ×70,000.

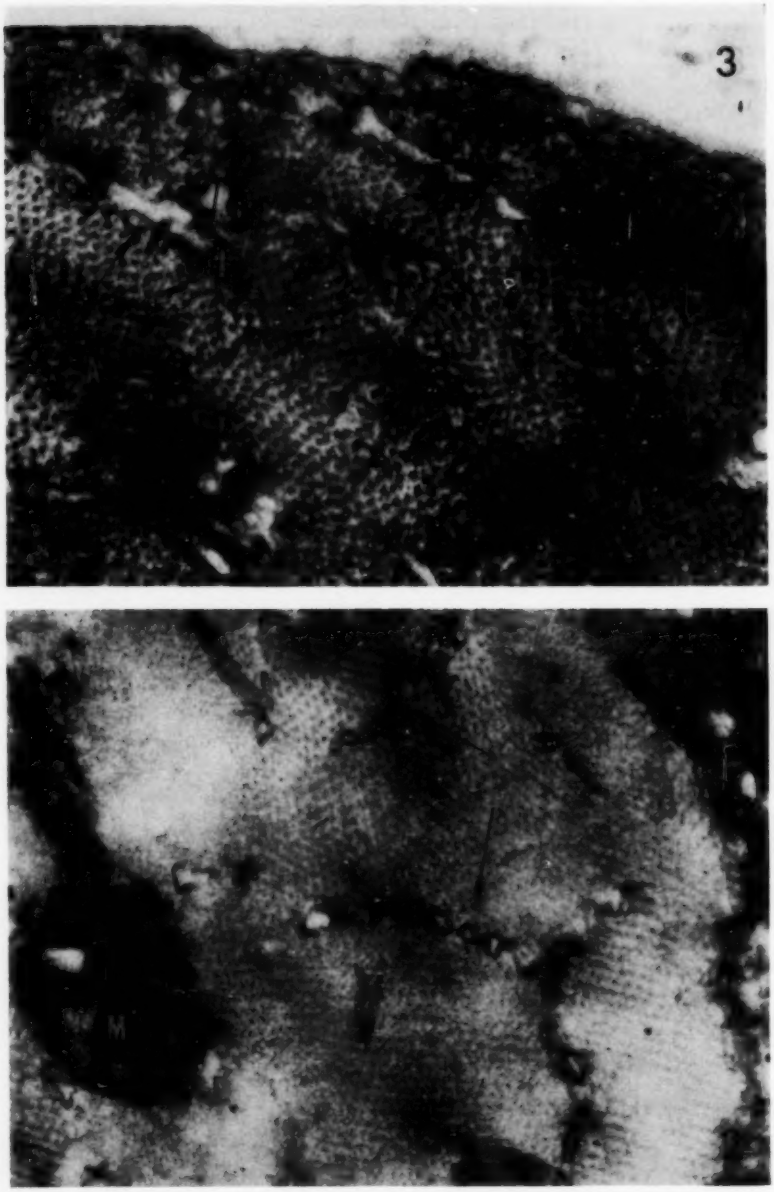

FIG. 2. Transverse section of striated muscle. Normal control. Vesicular elements of the sarcoplasmic reticulum are indicated by arrows and the mitochondrion by the letter M. ×80,000.

FIG. 3. Transverse section of striated muscle. Normal control. Tubular and vesicular elements of the longitudinal sarcoplasmic reticulum indicated by large arrows. The letter A designates the A band while the letter I designates the I band in cross section. A small arrow located above the white letter I indicates the transverse reticulum linking adjacent Z bands. Contrast of specimen enhanced by phosphotungstic acid. ×95,000.

FIG. 4. Longitudinal section of striated muscle in which the longitudinal elements of the sarcoplasmic reticulum appear to be clearly tubular (designated by large arrows). Normal control. The Z band area is indicated by the letter Z and a mitochondrion by the letter M. Small arrows at the right of the print indicate interfibrillar elements of the transverse reticulum. ×50,000.

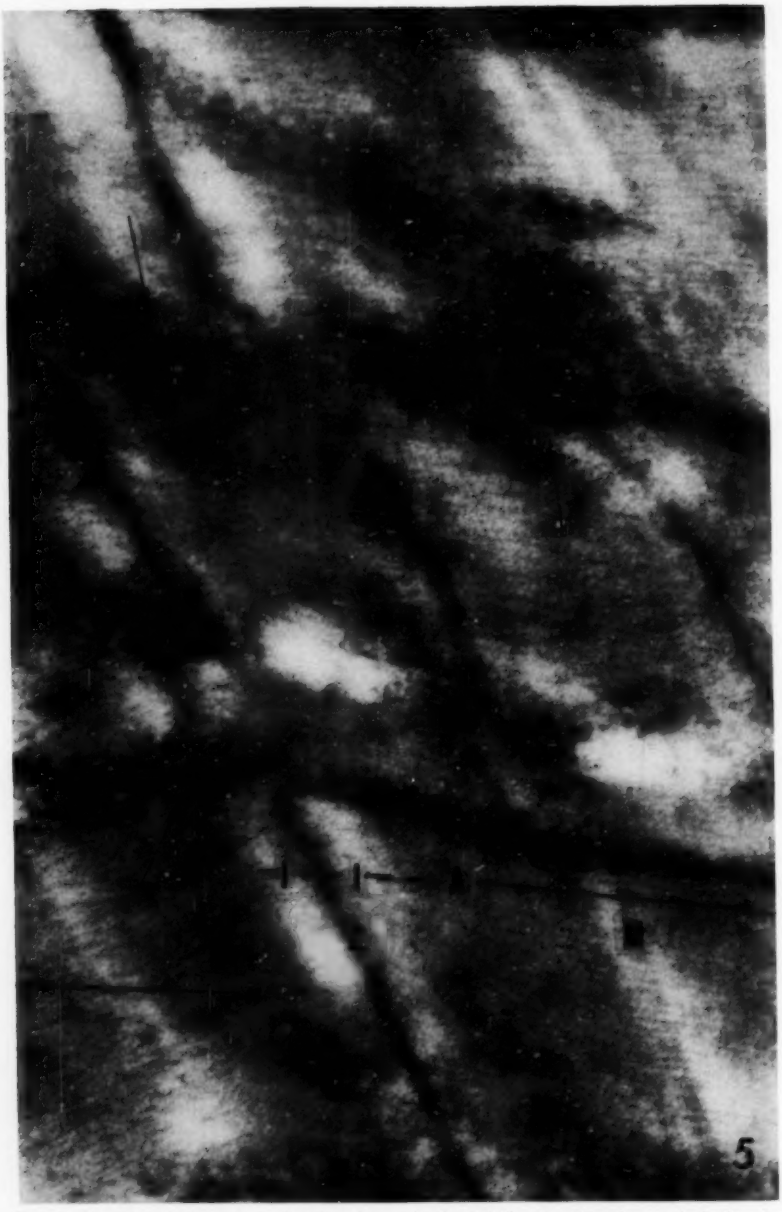

FIG. 5. Longitudinal section of striated muscle fixed following 30 minutes isolation from vascular and nervous supply. Although the amplitude of the muscular contraction (maximal response) remained normal, the longitudinal reticulum has become diffuse and granular (indicated by large arrows). The characteristic banding of striated muscle is indicated by appropriate letters and small arrows. ×70,000.

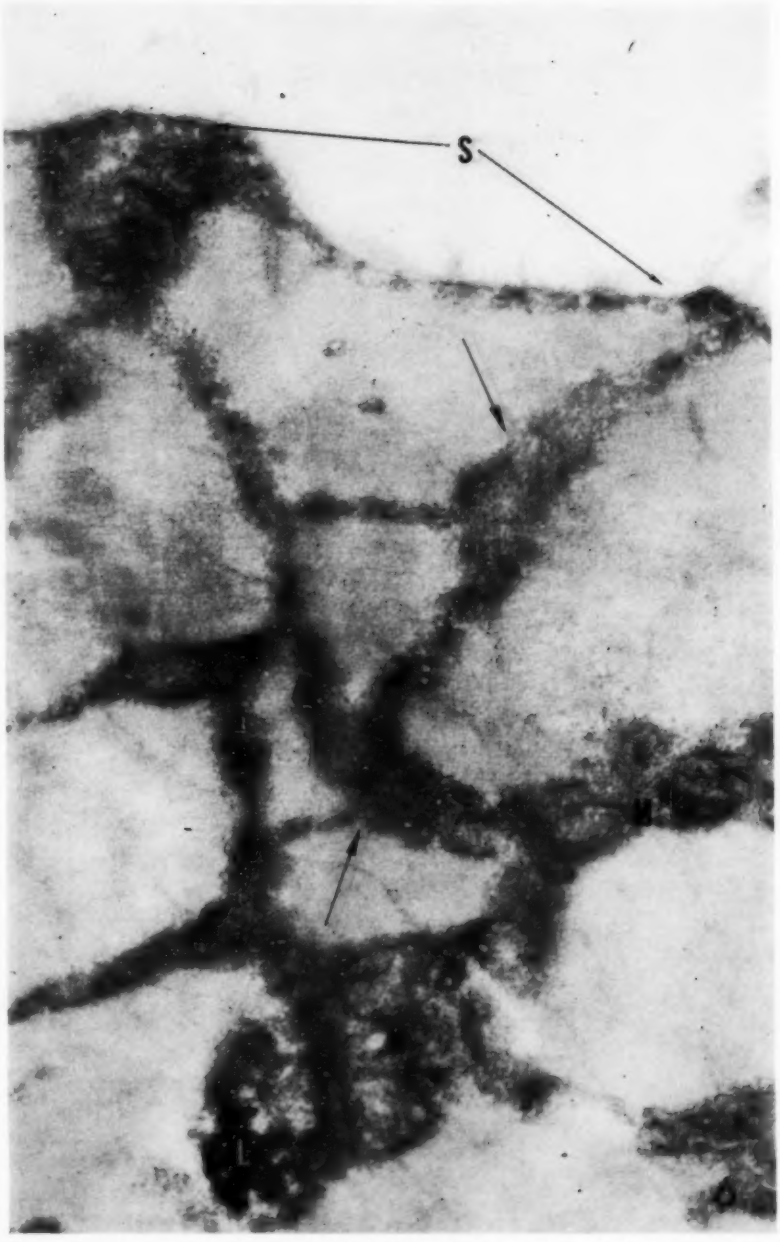

FIG. 6. Section of striated muscle typical of the 45 and 60 minute isolation. No vesicular or tubular elements can be recognized as the reticulum has become diffuse and granular (see arrows). Embedded in this matrix a lipid droplet (L) and abnormal mitochondria (M) can be identified. The sarcolemma is indicated by the letter S. ×25,700.

FIG. 7. Longitudinal section demonstrating the marked alteration in the longitudinal reticulum subjected to the 45 minute isolation from vascular and nervous supply. Arrows indicate abnormal mitochondria embedded in the markedly altered reticulum. ×67,000.

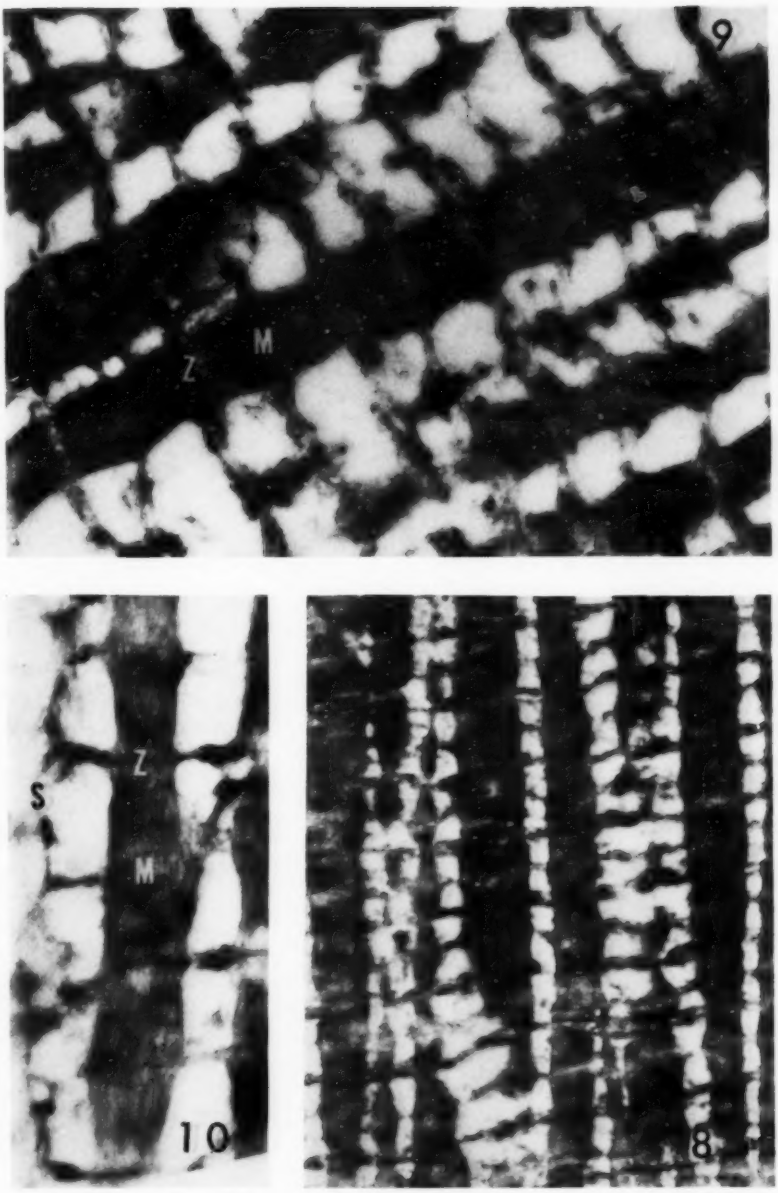

FIG. 8. Glycerol extracted striated muscle in longitudinal section. The transverse elements persist while the longitudinal components, mitochondria, and lipid droplets have been extracted. Transverse elements of the reticulum extend across the interfibrillar space linking adjacent myofibrils at the Z and M bands. A less conspicuous transverse element is occasionally seen linking adjacent N bands. ×10,000.

FIG. 9. Glycerol extracted muscle similar to Figure 8 but at higher magnification. The Z and M band areas are indicated by appropriate letters. ×19,500.

FIG. 10. Glycerol extracted frog sartorius muscle demonstrating the transverse elements linking the sarcolemma (S) with the Z and M bands (Z and M). The longitudinal elements of the reticulum have been extracted. ×21,000.

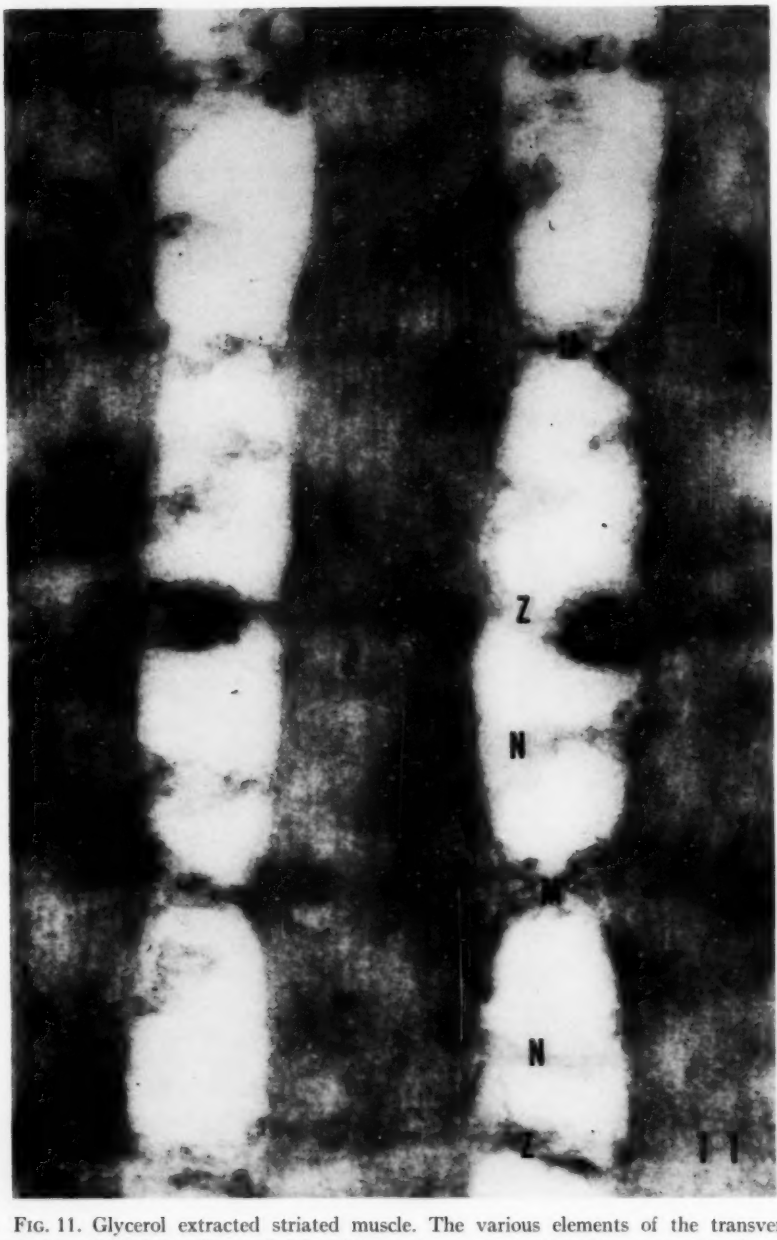

FIG. 11. Glycerol extracted striated muscle. The various elements of the transverse reticulum which join adjacent Z, M and N band areas are indicated by appropriate letters. ×71,250.

components of the reticulum are closely associated, they do not appear to be continuous. The longitudinal components are interrupted by the transverse reticulum at the level of the Z and M bands. Figures 1, 2, 3, and 4 demonstrate the sarcoplasmic reticulum in both longitudinal and transverse sections of normal (control) muscle.

Figure 5 is typical of the observations of muscles isolated from their vascular and nervous supply for a period of 30 minutes. The contractile apparatus remains similar to the normal controls. The longitudinal components of the sarcoplasmic reticulum have become primarily granular and diffusely organized. Figures 6 and 7 show the marked changes typical of the 45, and 60 minute isolation (10 specimens from 2 animals for each experimental period). The amplitude of the muscular contraction remained essentially normal although the muscles fatigued more rapidly upon repetitive maximal stimulation (approx. 10 v., 4/sec.) than the muscles stimulated for only 30 minutes.

Since the transverse components of the reticulum are not readily demonstrable in the experimental animals, it is likely that the generalized diffuse changes in the longitudinal reticulum mask this component inasmuch as they are normally very closely associated. Within the diffuse matrix of the longitudinal reticulum abnormal mitochondria and lipid droplets are recognizable (Figs. 6 and 7).

In glycerol extracted frog sartorius muscles the longitudinal reticulum, mitochondria, glycogen, lipid droplets and sarcoplasm are no longer demonstrable. Figures 8, 9, 10, and 11 clearly reveal the persistent, glycerol stable transverse reticulum linking the Z and M bands both to adjacent myofibrils and to the sarcolemma. The transverse reticulum linking the Z and M bands is not distinguishable from the sarcolemma or cell membrane. A delicate transverse structure joins adjacent N bands but has not been seen linking the N band with the sarcolemma.

DISCUSSION

The observations reported in this paper suggest that the morphological and functional concepts of the sarcoplasmic reticulum in striated muscle should be reinvestigated in a wide variety of muscles from various species. In addition to mere morphological considerations experimental analyses by chemical and physiological techniques must be undertaken if the role of these cytological components is to be elucidated.

The morphology of the reticulum was demonstrated in the early observations of Thin (1), Retzius (2) and others. These observations indicated that the transverse elements of the reticulum link the Z, M, and N bands to adjacent Z, M, and N bands and to the sarcolemma. In addition, longitudinal members of this network were described and Retzius (2) suggested that the whole three dimensional network might participate in the transmission of excitation within the muscle fibers. The intimate relationship of the reticulum

to mitochondria was also recorded. From the early 1900's little has been added to our knowledge of the distribution of this complex network, even with the aid of the electron microscope. The additional magnification and resolution on the electron microscope has shown the network to be primarily tubular with longitudinal and transverse components intimately associated.

Numerous investigations have been published (cited in the introduction) which seem to support the hypothesis first suggested by Retzius, namely, that the sarcoplasmic reticulum most probably serves in the transmission of excitation within the muscle fiber. A metabolic role has been suggested by at least two of the authors (9, 10) as an alternative hypothesis.

The results recorded in this paper suggest that the transverse element of the reticulum is markedly different from the longitudinal component. The transverse elements, sarcolemma and myofibrils, are glycerol stable while the longitudinal elements are extractable. The transverse elements alone link the sarcolemma to the myofibrils and the myofibrils with each other at the level of the Z, M and probably N bands. Inasmuch as the postmortem experiments indicate that the longitudinal component is labile it is probable that it is not essential for conduction, and if it is assumed that a sarcolemmal myofibrillar linkage is necessary, it seems inescapable that the transverse reticulum alone is essential for the internal excitation of the muscle fiber. It is premature to postulate a mechanism for the internal activation, but the evidence reported here that the transverse reticulum is not tubular (i.e., not phase separating) indicates that it cannot be explained easily in terms of an internal propagated impulse similar to sarcolemmal conduction as suggested by Ruska, *et al.*, (15). Since the sarcolemma is joined to the myofibrils by the transverse reticulum, the excitation carrier could be enzymatic, perhaps an electron transferring enzyme system. This could also be true for transfibrillar activation of the myofilaments. As it is known that the myofilaments are linked to each other, a continuous enzymatic system could exist across the muscle cell which is linked functionally to the sarcolemma. Huxley's observation (8) that the Z band area is important in internal excitation should also be interpreted to include the transverse component of the reticulum. What the role of the transverse M and N band reticulum may be is not yet clear.

Speaking against the role of the longitudinal components in conduction is the fact that it is discontinuous, being interrupted at the Z band by the transverse reticulum. The longitudinal component of the reticulum is unstable in glycerol as are the mitochondria, lipid droplets and glycogen. It is also interesting that the longitudinal reticulum is intimately associated with organelles and inclusions known to be related to the metabolic activity of the cell. Since conduction and excitation can occur in isolated muscles even after extensive postmortem changes in the longitudinal reticulum and mitochondria, their

role in internal activation can only be indirect. The variability noted in the morphology of the longitudinal reticulum from a tubular to a diffusely organized granular matrix in normally conducting and contracting cells suggests it could not serve with any efficiency a role in conduction in such a highly organized functional system. Morphological lability is not characteristic of any known conducting system.

Since the transverse elements of the sarcoplasmic reticulum are relatively inconspicuous even in well-fixed tissue, it would appear that many morphological conclusions and comparative functional correlations have been based primarily on the quantity and form of the longitudinal reticulum (6, 12). In the light of the observations in this study it is necessary to reevaluate earlier observations in terms of the dual nature of the sarcoplasmic reticulum and the particular sensitivity of the longitudinal component to metabolic stresses.

It would also seem desirable to revise the nomenclature to distinguish these two basically different cellular components. Since the longitudinal reticulum most closely resembles the reticulum of other cells and is located only in the subsarcolemmal and interfibrillar spaces, the term transverse myofibrillar bridges is suggested for the transverse component as it suggests a morphological and functional relationship.

Although many morphological features of muscle cells have been demonstrated they remain unexplained chemically and physiologically. As our knowledge is far from complete morphologically, chemically, and physiologically, any hypothesis related to the function of cytological components in muscle must be regarded as still speculative.

SUMMARY

1. The sarcoplasmic reticulum in frog striated muscle is composed of two distinctly different elements, i.e., a longitudinal, glycerol extractable component and a transverse, glycerol stable component.

2. The transverse component joins the sarcolemma to the myofibrils at the Z and M bands, and these areas of adjacent myofibrils to each other. Occasionally N to N band linkages are also recognized.

3. The longitudinal component is interrupted by the transverse component and is associated with mitochondria, glycogen, and lipid droplets. It is not continuous with the transverse component.

4. Presumably, conduction and muscular contraction can occur in spite of a radical postmortem change in the longitudinal component, i.e., from a well defined tubular system in normal controls to a granular, diffuse nontubular system in muscles which have been isolated from their vascular and nervous supply for 30 to 60 minutes.

5. Because of the stability of the sarcolemma, the transverse components

and the myofibrils in glycerol extracted muscle, it is tentatively suggested that the transverse components are in some manner associated with myofibrillar activation and contraction.

6. Since the longitudinal component is associated with mitochondria, lipid and glycogen, all of which are extractable in glycerol, and inasmuch as the mitochondria and longitudinal component undergo radical morphological changes without affecting muscular contraction, it is suggested that the longitudinal component serves some metabolic function not directly related to myofibrillar activation.

7. Because of the marked differences demonstrated in the two components of the reticulum, it is proposed that the term sarcoplasmic reticulum be retained for the longitudinal component, and the term transverse myofibrillar bridges be applied to the transverse component.

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DECONTAMINATION STUDIES WITH THORIUM X, AND A NOTE ON ITS PROBABLE MODE OF ACTION

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Thorium x was introduced into dermatologic therapy by Naegeli and Jessner (1) in 1913, and has been gaining increasing acceptance in this country as a source of alpha radiation since 1938 (2). There have been many reports (3–10) concerning its clinical use, and extensive carefully-planned studies of its mode of action (11–13) have been published within the past seven years.

Thorium x, a decay product of mesothorium, is a naturally-occurring isotope of radium, and is therefore an alkaline earth metal. While more than 90 per cent of its energy emission is in alpha particles, appreciable amounts of beta and gamma rays are emitted. The range of the alpha rays in tissue does not exceed 90 microns. The beta rays are of somewhat lower average energy than are those of the radium series, and the gamma rays are slightly more penetrating than those derived from the decay of radium.

Although thorium x has been used as a source of alpha radiation for the skin since 1913, nothing could be found in the literature concerning contamination of the user's fingers, the problems of radiologic safety involved, or the proper disposal of radioactive residues. The promotional literature of the suppliers reflected this by suggesting that the handling of thorium x was completely safe.

At the first use of thorium x in this clinic in December, 1951, it was observed that the fingers holding immobile the eyelid to which the alcoholic solution was being applied were being contaminated. Immediate scanning of the washed fingers at the Isotope Center indicated considerable radioactivity, so it appeared desirable to examine the problems of protecting the operator's fingers and of removing from them thorium x which might contaminate them accidentally.

MATERIALS AND METHODS

Thorium x was used in solution in propyl alcohol, at strengths ranging downward from 150 microcuries (uc)/cc. These solutions were applied by vigorous swabbing to the palmar surfaces and to hairy and non-hairy areas on the dorsal surfaces of the fingers and hands, and the alcohol was allowed to evaporate before baseline counts were made. The hands were dried with paper towels after each washing procedure. Determinations were made by counting the beta emission with a Geiger tube and a decade scaler, at distances of 7 to 10 cm. from skin to end of tube. The Geiger tube had a mica window of 1.5 mg./cm_2.

Decontamination Procedure	*Percentage of Contamination Remaining*	
	Dorsa of Hands	Palmar Surfaces
Tap water, vigorous rinse	66%	77%
Two washes, toilet soap and water	61%	56%
Two washes, soap, followed by vigorous 95% ethanol rinse	61%	57%
Vigorous wash, 95% ethanol	93%	93%
Ethanol wash followed by soap scrub	61%	60%
Wash, anionic detergent, acid pH (Vel)	37%	65%
Wash, $BaCl_2$ solution	37%	59%
With Surgical Rubber Gloves on Hands:		
(Thorium x applied to gloved hand)		
95% ethanol rinse	71%	
Subsequent wash, soap and water	13%	
Hand, after removal of gloves	0.015%	

Residual contamination of the hands was followed for several days on two occasions. The disappearance curves had half-life values (corrected for radioactive decay) of 8 and 16 hours respectively. Dorsal and palmar disappearance curves were quite similar in the two tests.

RECOMMENDED TECHNIQUE FOR THERAPEUTIC USE

The working surface on which the thorium x is handled is covered with two layers of absorbent paper towelling backed by two sheets of hard-finish paper, to prevent accidental contamination by spillage. The operator opens the corrugated cardboard shipping box and the lead shipping jacket with bare hands, and then dons surgical rubber gloves. The glass vial containing the alcoholic solution of thorium x is opened with the gloved hands, and the vial is stood up in the open lead jacket. The solution is applied to the lesions with cotton applicators, which are dropped onto the paper towelling after use. The screw cap is replaced on the vial, and the rubber gloves are removed and dropped, inside out, on the paper towelling. The lead jacket is closed with bare hands and replaced in the cardboard shipping box. The paper towels are folded to enclose all of the contaminated materials, this wad is wrapped in the hard-finish paper, and the whole mass is placed in the cardboard box, which is then relegated to a remote corner of the clinic or office. The box remains there for at least a month, by which time the thorium x has gone through eight half-lives or more and is reduced to less than 0.6 uc/cc. The radioactive residua are then regarded as effectively inert and are discarded through ordinary channels. The gloves may be put back into use without material danger.

ASSESSMENT OF RISK

It seemed possible that this investigation might be without purpose, for no reference to bone marrow or other injury produced by thorium x in physicians using it could be found after forty years of clinical use. To clarify this point,

the total emissions as alpha, beta, and gamma rays occurring in the decay of thorium x to lead were calculated, in roentgens (r) or roentgen equivalents physical (rep), according to the method of Lea (15). These determinations assumed an application of 1 uc/cm_2. of skin, with no loss save by radioactive decay, and included only that portion (approximately half) of the emission which was directed toward the skin. The calculated total doses on the skin were 233,000 rep as alpha, 785 rep as beta, and 6.5 r as gamma rays. The doses at a depth of 88 microns, the level of greatest penetration of the alpha, were 29,000 rep, 500 rep, and 6.5 r, respectively.[1]

DISCUSSION

The calculated total emissions show that repeated absorption of thorium x by mouth, or of thoron gas by inhalation, might offer a definite threat to the bone marrow. A recent report by Wyard *et al.* (14) emphasized the danger of inhalation of thoron in a clinic where thorium x is used frequently, but did not touch on the possibility of absorption via the fingers-to-mouth route.

It is evident that the danger is not in the few exposures to which the patient is subject, for the half-life of thorium x is only 3.64 days; but rather in the oft-repeated contacts which the dermatologist using thorium x therapeutically would have with it over the years. It follows, then, that established principles of radiologic safety must be observed in handling the material.

None of the decontamination procedures tried was able to remove all, or even the bulk, of thorium x applied to the skin in an alcoholic menstruum. Soap and water reduced counts to 61 per cent on the dorsa of the hands and to about 57 per cent on the palms, the difference being scarcely of moment. An anionic detergent, used to obviate precipitation of the thorium x as an alkaline earth soap, and $BaCl_2$, used to displace the heavier alkaline earth ion, both brought contamination of the dorsa of the hands down to 37 per cent, confirming the holding of thorium x on the hair and loose skin as an insoluble soap. Counts on the palms remained high, though, in accordance with Witten's finding that thorium x got down into the sweat ducts, where it would be inaccessible to decontaminating agents. Whether thorium x was bound chemically on the skin surface or had penetrated, by virtue of the low surface tension of the alcohol, between and under cells of the horny layer was not shown by these experiments. The possible effect of a chelating agent applied prior to contamination was not examined.

The experiments with rubber gloves showed that most of the applied thorium x could be washed from the smooth, relatively impervious rubber with soap and water, and that virtually none of such contamination reached the skin. Since tube distances were varied so that counts remained at 10,000 or less

[1] The calculations were checked by Dr. Franco Rasetti.

per minute, the 0.015 per cent of contamination found on the hands after careful removal of the gloves was within the limits of experimental error.

Up to several years ago, the screw-top glass vials in which thorium x is shipped were capped with a plastic cover intended to hold the screw top in place. It was observed that this plastic cover occasionally became soft and loose from minute leakage past a not-quite-tight screw top, so that the cover was a radiologic hazard rather than a safeguard. This was reported to the company supplying the thorium x, and the use of the plastic covers was discontinued.

The infrequent use of thorium x in this clinic is considered not to pose a hazard to the operator from inhalation of thoron, as discussed in the report (14) of the Guy's Hospital group. The ventilative procedures recommended by them should be carried out if thorium x were used more often. Their data suggest considerable inhalation of thoron by the patient when the face is treated.

INCIDENTAL CONSIDERATIONS CONCERNING THE MODE OF ACTION OF THORIUM X

The assessment of the risk involved in using thorium x proved to be a serendipitous exercise, for the calculated total dosages available as alpha, beta, and gamma radiation afford considerable food for thought. The low gamma emission, of high energy and at zero source-skin distance, can scarcely have any detectable effect in the skin. The beta emission is within the range of therapeutic beta dosage. Clinical application of thorium x usually exceeds 1 uc/cm_2., while the energy content and penetrating power of the beta are somewhat lower than those of the beta emitted by radium.

Witten *et al.* showed (13) that the beta-gamma components are capable of producing erythema and pigmentation, but assumed from their degree that the biologic effectiveness of these components was much less than that of the alpha. It is possible that the relatively mild reaction to beta-gamma observed by these workers was due to the low intensity of the beta, whose calculated magnitude is still adequate for the therapeutic effects observed. The calculated depth doses of total beta of thorium x are 146 rep at 1 mm., 94 rep at 2 mm., and 62 rep at 3 mm., assuming an application of 1 uc/cm_2. In comparison, a surface dose of 300 r of hard Grenz rays (17, 18), a fairly potent dose for ordinary dermatoses, would yield about 90 r at 1 mm., 42 r at 2 mm., and 21 r at 3 mm. depth.

The 233,000 rep of alpha on the skin surface, or even the 29,000 rep at 88 microns depth, is enough to stagger the imagination. The survival of the epidermis under so intensive a bombardment can mean only that this enormous energy is expended at a level well above the basal layer[3], and suggests that the

[3] The biologic and biophysical considerations expressed here were approved by the late Dr. Jonas Friedenwald and by Dr. Russell H. Morgan.

alpha radiation must be therapeutically inert, save for that small fraction which Witten's radioautographic studies (11, 12) show to be liberated in the hair follicles and sweat ducts. The conclusion appears inescapable that thorium x is not an effective agent for alpha therapy, and that the observed therapeutic effects are expressions of protracted beta radiation.

This conclusion is susceptible of experimental proof, and cannot be accepted finally without such proof. It will be necessary to irradiate living human or appropriate animal skin with a beam of alpha particles of 8–10 Mev. A reaction in any way approaching clinical magnitude would mean only that therapeutic effects from the alpha of thorium x are possible, but failure of such a reaction to occur would preclude the possibility of therapeutic activity from the alpha of thorium x. A particle accelerator of adequate power may some day become accessible. . . .

In the clinical use of thorium x, the skin is not exposed to the full calculated doses of alpha, beta, and gamma. The disappearance curves show that applied thorium x is lost rapidly from the hands, where the traffic is heavy. It is to be expected, then, that much of the applied thorium x must be lost from even highly protected areas through the frictions of everyday life. Covering of the treated areas with flexible collodion, as is sometimes done, might delay this loss for a day or two, but might also provide picking fingers with a target.

SUMMARY AND CONCLUSIONS

1. Experimental investigation of some of the problems of radiologic safety involved in handling and using thorium x was undertaken.
   a. It was not possible in these experiments to decontaminate adequately the purposely contaminated hands.
   b. Surgical rubber gloves protect the operator's hands completely from thorium x in alcoholic solution.
   c. Strict observance of the principles of radiologic safety is called for in the use of thorium x.
   d. A safe technique, as developed in this clinic, for therapeutic application of thorium x and for disposal of radioactive residues is presented.
2. Calculations of the total dosages available as alpha, beta, and gamma radiation suggest that the alpha emission of thorium x must be therapeutically inert.
3. The observed therapeutic effects of thorium x probably result from protracted beta radiation.

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NEUROLOGICAL DISORDERS IN AORTIC STENOSIS

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Attacks of syncope occur frequently in patients with aortic stenosis. While the association was probably first mentioned by Cowper in 1721, (1) it was not until the 1930's that physicians became aware of its relative frequency (2, 3, 4, 5, 6, 7). Other authors (8, 9, 10) have generally agreed that the association is a real one. Although Hammersten (10) and Marvin and Sullivan (5) mention the occurrence of convulsions concomitant with syncopal attacks in this syndrome, this association has not been described by most other authors. The purpose of this survey is to determine the frequency of syncope, convulsions, and other neurologic complaints in patients with aortic stenosis in this hospital.

MATERIAL

A total of 326 patients was used in this study. The records were obtained from two sources. The adult cases of aortic stenosis, 181 in all, were admitted to the wards of the Johns Hopkins Hospital during the period from 1946 to 1956. Of these, 25 underwent aortic valvulotomy. An additional 145 cases in children were seen in the Cardiac Clinic of the Harriet Lane Home from 1947 to 1958. Most of these were also admitted for cardiac evaluation. The hospital records of all patients were surveyed carefully with particular reference to the points in question. Certain neurological manifestations, including syncope, convulsions, dizziness, headache, hemiparesis, and deafness were found in 63 of the 326 patients. The frequency and severity of these neurological abnormalities were different in patients with congenital heart disease as compared with those found in acquired forms of aortic stenosis. The cases have therefore been divided for descriptive purposes into two groups:

Group I: Patients with congenital aortic, or subaortic stenosis.

Group II: Patients with acquired aortic stenosis.

RESULTS

It is evident that certain indications of neurologic disorder, including syncope, convulsions, hemiparesis ran throughout the records of both groups. For clarity, the distribution of patients with different complaints in each group is shown in Table I. A few isolated neurological complaints, such as

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TABLE I

Distribution of Patients with Neurological Complaints

	Group I	Group II	Total
Seizures	5	9	14
Syncope	10	12	22
Dizziness	3	2	5
Hemiparesis	0	7	7
Deafness	3	0	3
Other	9	3	12
Total	30	33	63
Total population	133	193	326
% of total population	23	16	19

deafness and congenital diplegia were clearly associated congenital abnormalities. Certain of these disorders can, however, be considered separately in somewhat greater detail.

1. *Seizures*

The distinction between syncope and seizures was far from clear. It was arbitrarily decided therefore to define seizures as those episodes of unconsciousness associated with convulsive movements and/or sphincter incontinence. Electroencephalography was performed in so few patients that comparative results were of no significance.

The clinical data concerning the 14 patients with seizures are listed in Table II. Five of these patients were children in whom the relationship of the attacks to cardiovascular symptoms could not be established with any degree of accuracy. Nine, however, were adults with acquired aortic stenosis. All but one of these had at one time or another dyspnea, cardiomegaly, or other symptoms or signs attributed to severe narrowing of the aortic valve, but the relationship of these to the appearance of seizures was doubtful. It is true that in three patients, the appearance of convulsions came after the onset of severe symptoms of cardiovascular insufficiency and were often related to exertion. On the other hand, 6 patients had their first convulsions before any cardiac symptoms had developed. Moreover, in 5 of these, there was never any indication that exertion was a factor in precipitating the attacks.

2. *Syncope and dizziness*

Syncopal attacks, in contrast to seizures, were arbitrarily defined as those episodes of unconsciousness not associated with convulsive movements and/or sphincter incontinence. There were 27 patients with such attacks. These are described in Table III.

It can be seen, that both with congenital and acquired aortic stenosis, the

attacks were frequently precipitated by moderate or severe physical exertion. Of the children with congenital lesions, syncope or dizziness appeared for the first time in half the cases before any cardiac symptoms were apparent. In contrast, of the adults with acquired aortic stenosis, only three were without complaints referable to cardiac insufficiency at the time of the first syncopal attack. Moreover, it should be noted that syncopal attacks ceased after operation in all three patients who were subjected to valvulotomy.

3. *Hemiparesis*

Hemiparetic signs were present in seven patients, all with acquired aortic lesions. Three of these were embolic in origin in young patients with subacute bacterial endocarditis or auricular fibrillation. Three more occurred in older persons and may well have represented cerebral vascular disease. One man of 31 years experienced solitary transient hemiparesis during an episode of unexplained tachycardia.

4. *Deafness*

Three pediatric patients with mild aortic or subaortic stenosis suffered from congenital deafness. None of these patients had any symptoms apart from hearing difficulties. They were being followed by the Cardiac Clinic for heart murmurs heard at an early age.

5. *Miscellaneous*

Twelve patients had neurological complaints not included in the preceding categories. Headache was the principal complaint in 5 persons. The headaches were always recurrent with migrainous features. Since none of these patients had severe cardiovascular disability and since the headaches were not related to exertion, the symptom was thought not to be causally related to aortic stenosis. Other miscellaneous disorders were one case of mental retardation, one of spastic diplegia, two cases of "falling spells" unaccompanied by unconsciousness, in young children, and two people with retinal artery occlusions. None of these patients had cardiac symptoms.

DISCUSSION

It is clear in this series that neurological complaints are not uncommon in patients with stenosis of the aortic valve. To assume a cause and effect relationship would seem unwarranted in many of the cases. The hemipareses, for example, were in most cases easily attributed to coexisting cerebrovascular disease. Past authors have considered the syncope occurring in patients with aortic stenosis as due to cerebral anoxia, directly or indirectly caused by the narrowed aortic valve. The rather large number of patients in this present series with syncope, and the frequent alleviation by surgery, would likewise

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Patient Number	JHH Number	Sex	Age at onset of neurological complaint	Age at onset of cardiac symptoms	Type of heart lesion	Valvulotomy	Neurological disorder	Frequency of attacks	Duration of attacks	Asys	Ankle swelling	Dyspnea
1	B7942	M	1 2 5	—	Congenital aortic stenosis	0	a. Syncope. b. Convulsions. c. Headache (migraine).	Frequent. Occasional. Frequent.	Seconds. Seconds.	0 0 0	0	+ —
2	B2576	M	13 21	—	Probable aortic stenosis. ?Pulmonary stenosis.	0	a. Unconsciousness. b. Unconsciousness with incontinence.	Rare. Rare.	— Few minutes.	—	0	0
3	A83568	F	9 months	—	Minor subaortic stenosis.	0	Grand Mal epilepsy.	Frequent.	—	—	0	0
4	A98649	M	3	—	Subaortic stenosis.	0	Generalized convulsion following fever.	Rare.	Several hours.	—	0	0
5	508472	M	9	2	Patent ductus with subaortic stenosis.	0	Generalized clonic convulsion after oral antihistamine.	Rare.	About 30 minutes.	0	0	++
6	531344	M	19 22	19	Calcific aortic stenosis.	+	a. Fainting spells. b. Spells with incontinence, and kicking of feet.	Occasional.	4-5 minutes.	0 +	0	+
7	418602	F	51	49	Calcific aortic stenosis.	+	Convulsion.	Rare.	Several minutes.	—	0	+
8	758934	F	50	49	Calcific aortic stenosis; ?rheumatic heart disease.	+	Fainting and convulsions with incontinence.	Frequent.	5-10 minutes.	+	+	++
9	474789	M	22	29	Hypertensive cardiovascular disease; rheumatic heart disease with aortic insufficiency, aortic stenosis, mitral insufficiency.	0	Uncinate seizures; hemiparesis.	Frequent.	—	0	+	+
10	506951	F	44 49	53	Aortic stenosis; Adams-Stokes.	0	a. Fainting spells. b. Generalized convulsion.	Occasional. Occasional.	Minutes-hours. minutes.	—	0	+
11	399127	F	37	—	Rheumatic heart disease with aortic stenosis, mitral stenosis.	0	Epileptic spells, generalized.	Frequent.	20 minutes.	—	0	0
12	767864	M	55	—	Calcific aortic stenosis.	0	Generalized convulsions.	Occasional.	5-10 minutes.	+	0	0
13	488866	M	6	31	Aortic stenosis and insufficiency, mitral stenosis and insufficiency, congestive heart failure.	0	Convulsions from age 6-13 after "spinal meningitis".	—	—	—	++	+++
14	430844	F	Childhood	32	Calcific aortic stenosis; mitral stenosis; congestive heart failure.	0	Grand Mal epilepsy.	—	—	—	—	+++

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ta on Pa Convulsions
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ation of tacks	Aura	Ankle swelling	Dyspnea	Thrill	Cardiomegaly	EKG	Blood pressure	Autopsy	Remarks
onds. onds.	0 0	0	+ —	+	0	Left axis deviation; slight left ventricular hypertrophy.	Normal.		No symptoms for 3 years. Given phenobarbital.
— r min- tes.	– –	0	0	+	+++	Left axis deviation; left ventricular hypertrophy; Wolf-Parkinson-White syndrome.	Normal.		None.
—	–	0	0	0	0	—	Normal.		None.
eral ours.	–	0	0	+	Slight	Focal intraventricular block.	Normal.		None.
out 30 inutes.	0	0	++	++	+	Slight left ventricular hypertrophy.	Normal.		Prescribed with digitalis for congestive heart failure; no further convulsions after 3 months.
min- tes.	0 +	0	+	+	+	—	Normal.	None.	Patient died of ventricular fibrillation after valvulotomy.
eral ninutes.	–	0	+	+	+	Left ventricular stain.	Normal.		Discharged improved after valvulotomy.
0 min- tes.	+	+	++	–	++	Left ventricular hypertrophy.	Normal.		Cerebrovascular accident, mild, after valvulotomy.
—	0	+	+	+	+	—	230/60–90	Rheumatic valvulitis. Glomerulonephritis.	Died in failure at 29 years.
inutes- hours. 25 minutes.	–	0	+	0	+	Right bundle branch block; complete heart block.	165/65	Arteriosclerotic cardiovascular disease. Aortic valve 5.8 cm. Narrowed left carotid; bilateral encephalomalacia.	Died at 53 in failure.
minutes.	–	0	0	++	+	Digitalis effect.	130/95	None.	Died at 52 of cerebral embolus.
10 min- utes.	+	0	0	0	0	Moderate left ventricular hypertrophy.	Normal.		Improved on phenobaribtal and activity restriction.
—	–	++	+++	+	++	—	Normal.	Extreme aortic stenosis; aortic valve—6 cm.	Died in congestive heart failure at 31 years.
—	–	–	+++	–	+++	Left ventricular hypertrophy.	Normal.	Marked aortic stenosis; aortic valve 3–4 cm.; mitral, bicuspid stenosis.	Died in congestive heart failure. Epilepsy well-controlled with Luminal.

TABL

Data on Patients ... *and Di*

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Patient Number	JHH Number	Sex	Age at Onset of Neurological Complaint	Age at Onset of Cardiac Symptoms	Type of Heart Lesion	Valvulotomy	Neurological Disorder	Frequency of Attacks	Duration of Attacks	Aura	Ankle Swelling	Dyspnea
1	A77019	F	3	—	Congenital aortic stenosis.	0	? Petit Mal attacks.	Occasional.	4–5 minutes.	–	0	0
2	B9460	M	8	13	Congenital aortic stenosis.	0	Syncopal spells; Migraine.	Occasional.	10–15 minutes.	–	–	++
3	B23606	F	11	0	Congenital aortic stenosis.	0	Syncope.	Occasional.	—	–	0	0
4	A72141	F	2	1	Congenital aortic stenosis.	0	Syncope in early childhood.	—	—	–	0	+
5	B2312	M	7	—	Congenital aortic stenosis.	0	Fainting spells.	Rare	5 minutes.	–	0	+
6	A77690	M	6	12	Congenital aortic stenosis.	+	Giddy spells; blackout spell.	Occasional.		–	0	++
7	B21027	M	8	—	Aortic stenosis and aortic insufficiency.	0	Dizzy spells; syncope.	Rare.	15 minutes.	+	0	0
8	A89625	M	11	—	Coarctation; subaortic stenosis.	0	Syncope; dizziness.	Occasional.	3 minutes.	+	0	0
9	B28860	F	10	—	Aortic stenosis; coarctation.	0	Fainting spells.	Rare.	—	+	0	0
10	A69396	F	5	—	Pulmonary stenosis; ? Aortic stenosis.	0	Fainting spells.	Rare.	—	–	0	0
11	214409	M	62	60	Calcific aortic stenosis.	+	Loss of consciousness; dizziness.	Occasional.	2–3 minutes.	–	+	+
12	764820	M	41	43	Calcific aortic stenosis.	+	Syncope.	Frequent.	—	–	0	+
13	721623	M	20	17	Calcific aortic stenosis.	+	Syncope.	Occasional.	10 minutes.	–	0	+
14	679031	M	51	47	Calcific aortic stenosis.	+	Syncope.	Rare.	20 minutes.	–	0	++
15	758813	M	43	35	Calcific aortic stenosis.	+	Syncope.	Frequent.	—	+	0	+
16	734185	M	49	49	Calcific aortic stenosis	+	Syncope.	Frequent.	2–5 minutes.	+	0	0
17	730361	F	66	66	Calcific aortic stenosis	+	Syncope.	Occasional.	—	+	0	0
18	501523	M	57	54	Calcific aortic stenosis; mild aortic insufficiency.	0	Syncope.	Rare.	Minutes.	+	+	+
19	349509	F	63	—	Calcific aortic stenosis; aortic insufficiency.	0	Syncope; dizziness.	Occasional.	Minutes.	–	0	0
20	A64182	M	40	35	Calcific aortic stenosis; minimal aortic insufficiency.	0	Syncope.	Frequent.	Minutes to one hour.	0	0	+
21	377829	M	61	54	Rheumatic heart disease with aortic stenosis and aortic insufficiency.	0	Unconsciousness.	Rare.	Minutes.	0	0	+
22	431694	F	11	11	Rheumatic heart disease with aortic stenosis, mitral stenosis, mitral insufficiency, subacute bacterial endocarditis.	0	Unconsciousness; giddiness.	Frequent.	30 minutes.	–	0	+
23	A84323	M	15	—	Subaortic stenosis; marked aortic insufficiency.	0	Dizziness.	Occasional.	—	–		
24	A79287	F	7	—	Patent ductus arteriosis (ligated); aortic stenosis.	0	Dizzy spells.	Occasional.	—	–	0	+
25	A73385	F	20	20	Subaortic stenosis; small interventricular septal defect.	0	Dizzy spells.	Frequent.	5–20 minutes.	–	0	0
26	467981	M	44	39	Aortic stenosis.	0	Dizzy spells.	—	—	–	0	0
27	451832	F	13	17	Rheumatic heart disease with myocarditis, mitral stenosis, mitral insufficiency, aortic stenosis, aortic insufficiency, auricular fibrillation.	0	Dizziness.	Occasional.	Frequent.	–	0	+
											+	+

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...kish Spelling	Dyspnea	Thrill	Cardiomegaly	EKG	Blood Pressure	Autopsy	Remarks
0	0	—	Slight.	Left ventricular strain; left ventricular hypertrophy.	88/80		No attacks for 2 years on limited exertion.
–	++	0	+	Left ventricular hypertrophy; strain.	—	None.	Patient died in congestive heart failure at 13 years.
0	0	++	0	Left ventricular hypertrophy.	Normal.		No spells for 2 years on mild activity restriction.
0	+	+	+	Left axis deviation; left ventricular hypertrophy.	Normal.		No attacks from age 12-21 years; patient has avoided suddenr exertion.
0	+	+	+	Left axis deviation; left ventricular hypertrophy.	Normal.		No attacks from 7-9 years on activity restriction.
0	++	+	0	Left ventricular hypertrophy.	Normal.		No spells several months after surgery.
0	0	+	0	Left ventricular hypertrophy.	112/18		None.
0	0	0	0	Left ventricular preponderance.	L.90/70 R.130/80		Coarctation repaired age 10. Spells began after operation.
0	0	++	0	Left ventricular hypertrophy; left ventricular strain.	Normal.		Spells absent for 1 year on restricted activity.
0	0	+	+	Right axis deviation; right ventricular hypertrophy with complete bundle branch block.	Normal.		Pulmonary valvulotomy at 6 years. No fainting for 1 year post-operative.
+	+	+	+	Left ventricular strain.	Normal.	Bicuspid aortic valve. Aortic stenosis: Valve-2 cm.	Died during valvulotomy.
0	+	0	++	Left ventricular hypertrophy.	130/100		No symptoms 2 months post-operative.
0	+	++	++	Left ventricular strain.	Normal.		Survived valvulotomy.
0	++	+	++	Digitalis effect.	Normal.	Calcific aortic stenosis of orifice. 1.5 × 0.8 cm.	Cardiac arrest during valvulotomy.
0	+	+	+	Left ventricular hypertrophy; strain.	126/98	Calcific aortic stenosis.	Cardiac arrest during valvulotomy.
0	0	+	0	Left ventricular hypertrophy.	Normal.		No symptoms 1 year post-operative.
0	0	0	+	Left ventricular hypertrophy.	120/90	Marked aortic stenosis; narrow right coronary.	Died at 67 after valvulotomy.
+	+	++	++	—	Normal.		Died at 61 in congestive heart failure. No syncope since 57.
0	0	0	0	—	150/78		Died of renal failure.
0	+	+	+	—	Normal.		None.
0	+	+	+	—	Normal.		Digitalized. No spells for 5 years.
0	+	+	+	Prolonged interval.	Normal.		Frequent anxiety attacks.
0	+	+	+++	Left bundle branch block.	Normal.		None.
0	0	+	0	Left ventricular hypertrophy.	Normal.		Dizzy spells 6 years after patent ductus arteriosis ligation.
0	0	+++	0	Left ventricular hypertrophy.	Normal.		Spells accompanied by tachycardia.
0	+	0	+	—	Normal.		None.
+	+	+	+++	Auricular fibrillation.	155/75	Old rheumatic fever. Slight aortic stenosis, mitral stenosis. Aortic valve 5.5 cm.	Died at age 20 in congestive heart failure.

seem to indicate a direct relationship to aortic stenosis. Mechanisms of production of the anoxia, however, are not clear. Incriminated amongst others have been:

1) Hyperactive carotid sinus (5).
2) Decreased cardiac output (4).
3) Lack of cardiac reserve and failure of left ventricular pressure to rise sufficiently during exertion (11).
4) Stokes-Adams attacks.
5) Arrhythmia.

No definitive information which might shed light upon the exact mechanism of production of the anoxia is available from the present analysis. However, the high incidence of angina pectoris in the adult patients (7 of 15) might well support the third mechanism proposed by Friedberg (11).

The present group showed a higher incidence of convulsions than previously reported by other authors. There may be several reasons for this. Firstly, the follow-up studies on many of these patients, particularly those in the pediatric age group were prolonged. Secondly, some of the patients, especially among those who underwent surgery, were referred to the hospital partly because of their convulsive episodes. Thirdly, the significance of tonic or clonic movements accompanying syncopal attacks is often not recognized. It seems reasonable to assume that the convulsions occurring in several of the cases reported here were also attributable to cerebral dysfunction resulting from aortic stenosis. Anoxia, caused by local cerebrovascular disease is a well-known cause of symptomatic epilepsy and probably played a role in these patients. Of special interest is the group of patients with seizures occurring before any symptoms of cardiovascular decompensation and having apparently no relation to exertion. It is possible that these patients were epileptic on the basis of factors unrelated to their cardiovascular abnormality. However, it may be that these patients had an abnormally low convulsive threshold and that their seizures were a subtle indication of cerebral dysfunction due to some impairment in cerebral blood supply. The treatment of patients with seizures is difficult to evaluate. The three patients with convulsions who underwent valvulotomy were not followed for a sufficient length of time to be certain about the benefits of operation in their cases. Similarly, information about benefits for medical treatment in these cases is meager. The three patients who were treated with anticonvulsant medication did, however, show symptomatic improvement.

Finally, it may be pertinent from a purely practical standpoint to emphasize that many of these patients first came to medical attention because of syncopal attacks or seizures. Their very numbers indicate the clinical importance of considering this form of cardiac disease as a possible underlying basis for symptoms such as these.

SUMMARY

1. A total of 326 cases with clinically recognized aortic stenosis was reviewed.

2. Sixty-three of the 326 patients were found to have neurological complaints as follows: seizures, 14; syncope, 22; dizziness, 5; hemiparesis, 7; congenital nerve deafness, 3; miscellaneous (including mental retardation, headache, and retinal artery occlusion), 12.

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RAEDER'S PARATRIGEMINAL SYNDROME

A Benign Disorder, Possibly a Complication of Migraine

F. R. FORD and F. B. WALSH

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In 1924, Raeder (1) published a paper in which he described a syndrome including drooping of the eyelid, and contracted pupil, i.e. signs of oculosympathetic nerve involvement, without loss of sweating on the face. He pointed out that the lesion must be at the base of the middle fossa involving the oculosympathetic fibers which lie in the wall of the internal carotid artery and first division of the fifth nerve. Five cases were given in abstract. A neoplasm was found in one. Two were due to trauma and the cause in two cases was obscure. In all cases there was evidence of involvement of the cranial nerves, especially the fifth nerve. This was termed the paratrigeminal syndrome. It was thought to have no etiological significance and was of value merely in localization of the process.

Lawton Smith (2) has recently published a very helpful study of Raeder's syndrome and gives eight case histories. Six of the eight patients had been followed for four years. He stresses the importance of distinguishing this condition from Horner's syndrome.

In recent years the writers have observed more than twenty-five cases of this syndrome in which only pain above the eye and the oculosympathetic phenomena were present. Males seem to be affected more frequently than females. The onset is, as a rule, in middle age or old age. The first symptom is a throbbing headache which is located above one eye or the other. It is invariably severe. The headache begins early in the morning and often awakens the patient from sleep. There may be nausea and vomiting associated. Often the pain will cease about noon. The pain occurs every morning and may persist for a number of weeks or even several months. As a rule, the headache is severe for only the first week or two.

Shortly after the headaches begin, the homolateral eyelid begins to droop and the pupil becomes smaller. The pupil does not expand in reduced illumination so the difference in the size of the pupils is more obvious in a darkened room. In a bright light, the difference in the pupils may be very slight. When cocaine is instilled the affected pupil does not dilate. The conjunctiva may be somewhat congested for a time. It seems clear, therefore, that the oculosympathetic nerve fibers have been damaged in some way. It should be pointed out that this is not Horner's syndrome for there is no loss of sweating on the face. Skin resistance to the galvanic current is not altered on the face. We agree with Raeder that the lesion must be at the base of the middle fossa. After

months or possibly a year, the lid lifts and the pupil expands to its normal size. We have observed a few cases in which a second attack occurred.

In our experience this syndrome is benign when it is uncomplicated. The presence of symptoms other than those mentioned above, vitiates the diagnosis and alters the prognosis.

The cause of this condition has never been established. Raeder apparently regarded it as merely an anatomical syndrome indicating a process at the base of the middle fossa. We are impressed with the stereotyped character of the syndrome and suspect that it is usually due to a specific cause. Aneurysm is almost always considered a plausible explanation but in our cases when arteriograms have been performed, no aneurysm has been found. We have never seen subarachnoid hemorrhage in our series of cases. We have never encountered an intracranial neoplasm.

When the patient is questioned carefully about headaches in the past, we have elicited almost without exception, a clear history of throbbing morning headaches of many years duration often associated with nausea and vomiting. We are, therefore, inclined to believe that Raeder's syndrome is merely a somewhat unusual result of a series of severe and frequent migrainous headaches. Dr. William Speed once suggested in conversation with one of the writers, that the mechanism by which the sympathetic nerve fibers is injured might be similar to that responsible for the third nerve palsy in ophthalmoplegic migraine. In both instances the signs develop only after prolonged headache and hence, are probably not caused in the same way as the visual phenomena and other symptoms which precede the headache.

Harold Wolff (3) has shown that in a migrainous attack the arteries become dilated and if the attack is prolonged the wall of the artery becomes edematous and thickened. The oculosympathetic nerve fibers lie in the sheath of the internal carotid artery where it lies at the base of the middle fossa. We suggest that these changes in the internal carotid artery may occasionally result in damage to the sympathetic fibers.

Horton (4) mentions that Horner's syndrome may occur in association with the type of headache which he has attributed to histamine sensitization. It is possible, however, he is dealing with the same condition we are describing.

It seems unnecessary to publish a series of case histories. They are all alike for the condition is a stereotyped one. We give merely one case history which is of interest because two attacks occurred, one on the left and then one on the right.

Case History (No. 733662) *Recurrent migrainous headaches for many years. At the age of 63 years, a series of severe headaches over the left eye with drooping lid and small pupil. Complete recovery. At the age of 65 years, a second episode identical with the first except for the fact that the symptoms were on the right.*

M. W. a man of 63 years was seen for the first time in 1956. He stated that for a great many years he had been subject to frequent headaches. These were frontal and located over one eye or the other. These started as a rule, early in the morning and would awaken the patient from sleep. The pain was of throbbing character. There was nausea but no vomiting as a rule. These headaches occurred at irregular intervals.

A few weeks before entering the hospital he began to have more severe headaches than ever before. The headache occurred every morning without fail. The character of the headaches was unchanged but the location was always over the left eye. Our examinations revealed only drooping of the left eyelid and a contracted left pupil. The pupil did not dilate in reduced illumination and did not respond to cocaine. There was no diplopia. No loss of sweating was found on the face. The headaches were beginning to diminish when the patient got to the hospital and soon were no longer a problem.

In March 1957, the patient was examined again. He was having merely an occasional mild headache at that time. The oculosympathetic signs were scarcely demonstrable.

Early in August 1958, the patient developed another series of severe morning headaches. These were identical with those which occurred in 1956 except for the fact they were over the right eye and not the left eye as in 1956. He noted that the right lid drooped and the right pupil was contracted. The conjunctiva was congested for a time. The patient entered the hospital on August 20. The headaches were beginning to abate at that time. The signs of oculosympathetic involvement on the right were obvious. The right pupil did not dilate when cocaine was instilled. There was no loss of sweating on the face. The left pupil had expanded to a proper size and the left lid no longer drooped. This patient was seen in consultation with Dr. R. Carmichael Tilghman.

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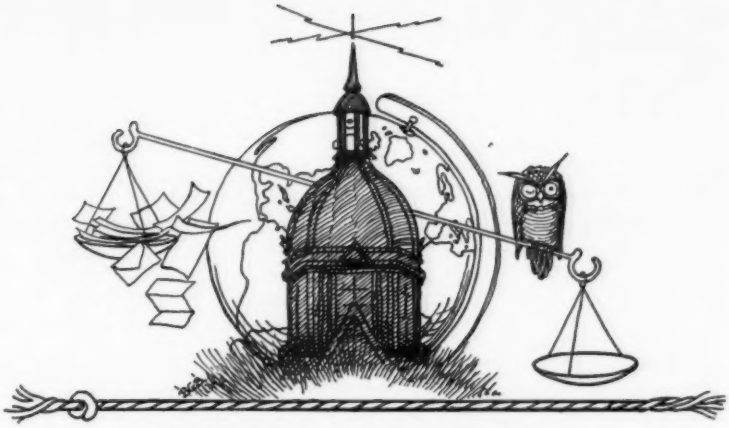

BOOK REVIEWS

Administrative Medicine. Transactions of the Fifth Conference Oct. 29, 30 and 31, 1958. Edited by GEORGE S. STEVENSON. 197 pp., $3.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

This is the fifth in a series of reports of conferences sponsored by the Josiah Macy Jr. Foundation which record the verbatim discussions of distinguished participants, directed toward uniformity in the administrative approach to a current problem in the multiprofessional field of health.

This conference considered the mental health movement in this country as its philosophy has altered as emphasis shifted from the mental hospital to the community health program with the increasing knowledge psychiatry contributed to recognition and prevention of mental illness. The report considers the relation of the mental hospital programs to the broad problems of medical and public health administration with specific reference to the relation of the mental hospital to the community and to the private practice of medicine. Anyone concerned with the administration of a mental health program will find reference to most of his current problems in the experiences discussed at this conference, and expert advice on the approach to solution.

HARRY L. CHANT

A Bibliography of Internal Medicine. Communicable Diseases. By ARTHUR L. BLOOMFIELD. 560 pp., $10.00. The University of Chicago Press, Chicago, Ill.

Most physicians and medical students familiar with Dr. Bloomfield's selected bibliographies that have appeared in the Archives of Internal Medicine and elsewhere will welcome this volume. In his effort to stress the historical approach to the study of disease, especially the scientific contributions of the last century, Dr. Bloomfield has reviewed the French, German and English language publications on thirty-one communicable diseases and chosen every reference that he believed of fundamental importance in the development of present-day knowledge of these diseases. These selected articles are presented in bibliographic form with carefully written, informative abstracts.

Nearly every reader will find areas in which he disagrees with the author's selections. Some may prefer a more conventional format for the presentation of medical history. Yet, all readers will admire the undertaking of such a gigantic task and be grateful to Dr. Bloomfield for providing so much valuable material in such a convenient form.

HENRY N. WAGNER, JR.

Atrial Arrhythmias, Digitalis and Potassium. By BERNARD LOWN AND HAROLD D. LEVINE. 222 pp., $6.90. Landsberger Medical Books, New York, N. Y.

The authors draw from their personal clinical and laboratory experience as well as from the literature to present an excellent review of the relationship between digitalis and potassium. The clinical section of the book is built around many illustrative case histories with electrocardiograms which have been reproduced in readable form. The physician practicing internal medicine will find the detail excessive but can profit by a brief perusal of the excellent summary paragraphs. The book will be useful as a reference source for cardiologists and physiologists. Stimulating speculation is presented regarding the interrelations of digitalis, acetylcholine and adrenal steroids at the cellular membrane.

RICHARD S. ROSS

Diagnostic Laboratory Hematology, 2nd edition. By GEORGE E. CARTWRIGHT. 250 pp., $6.75. Grune & Stratton, New York, N. Y.

It is refreshing to review a laboratory manual in which the author emphasizes the simplest and most reliable techniques in hematology. The procedures are clearly described, reagents identified, and selected references are given. Particular attention is paid to the sources of error and misinterpretation. A valuable feature is the brief statistical introduction and the illustrative examples of variation in results taken from student performances. The book is weakest when the author digresses from the laboratory procedures into brief summaries of physiology and clinical hematology. The written descriptions of marrow abnormalities are not helpful in the absence of accompanying illustrations or photographs. However the book effectively accomplishes the author's intention "to present in concise form the essential details for the performance and critical evaluation of procedures used commonly in student laboratories and doctors' offices for the diagnosis of hematologic disorders".

JULIUS R. KREVANS

Modern Clinical Psychiatry, 5th edition. By ARTHUR P. NOYES AND LAWRENCE C. KOLB. 694 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

The task of writing a textbook of Psychiatry is monumental. The complexity and diversity of material, the differing points of view, plus the many unproved developments, tax the judgment and editorial ability of any author. In the Fifth Edition of Dr. Noyes text, continuing his good works, he has been joined by Dr. Lawrence Kolb to produce an excellent system.

The emphasis in this volume is on sound clinical knowledge and principles. Where there is question or doubt as to the validity of theories or methods, the authors are careful to point out uncertainty. Too often psychiatrists become preoccupied with theories and overemphasis on psycho-pathology. This book stresses fundamentals, such as the observation of the patient, adequate history taking, careful mental status examination, consideration of dynamic formulations, review of mental mechanisms, and personality development. The main body of the book is devoted to the principal psychiatric disorders, those due to organic brain disease, or lack of physiological brain support, the psychoneurotic reactions, psychoses, psycho-physiological reactions, child psychiatry, and character disorders.

The chapter on treatment, although adequate in covering various methods, would be enhanced by specific examples of cases in which techniques and methods are illustrated. The authors devote a chapter to Pharmacology in which some of the more important new drugs are discussed with careful attention to complications and to proper use and especially to abuse of these agents. A section covering insulin and electroconvulsive therapy is informative to those not acquainted with these methods and useful for review to those who have not constantly practiced them. Finally, there is a new chapter devoted to the problems of psychiatry and the law.

In general, this is a first rate book, condensing within a seven hundred page volume a great deal of knowledge and carefully organized material. This reviewer would like to see more on therapy, particularly psychotherapy, a more extensive bibliography, and more information devoted to the psycho-physiological disorders.

This effort represents a constructive blend of the influences of Sigmund Freud and Adolph Meyer on American Psychiatry. The developments in the field are so fast moving and extensively written about in the literature that it would be well for any practitioner of psychiatry to read this book. It is certainly to be recommended to the medical student and to any person practicing medicine, as well as those working in allied fields.

WILLIAM T. DIXON

Hermaphroditism, Genital Anomalies and Related Adrenal Disorders. By HOWARD W. JONES, JR. AND WILLIAM WALLACE SCOTT. 456 pp., $16.00. The Williams & Wilkins Co., Baltimore, Md.

So broad in scope is this book that to many it may appear to be almost too ambitious an undertaking. However, Drs. Jones and Scott, a gynecologist and a urologist, respectively, have combined a vast clinical experience with a profound understanding of basic embryologic, anatomic, metabolic and psychologic concepts to produce a work which is of fundamental importance to those interested in the medical and surgical aspects of genital abnormalities. Initially, the book was inspired by Dr. Hugh H. Young's *Genital Abnormalities, Hermaphroditism and Related Adrenal Disorders*, published in 1937, but long in need of drastic revision. The present monograph has gone far beyond that goal by unifying in a thoroughly readable and understandable manner our current knowledge in this area.

The authors' personal experience with the subject has been augmented considerably by a long and intimate association with pediatric and adult endocrine groups at The Johns Hopkins Hospital. Through such contacts their surgical experience with genital abnormalities has become perhaps the broadest of our time.

The monograph is largely clinical in approach and the text is supplied with adequate case material which is described fully but not laboriously. Photographs, pathologic plates and line drawings are used liberally and are at all times beautifully reproduced. To enhance further the assets of the book are four chapters written by contributing authors: Dr. Alfred Jost, dealing with embryonic sexual differentiation; Drs. John Eager Howard and Claude J. Migeon, on Cushing's syndrome; Dr. Harry F. Klinefelter, on Klinefelter's syndrome; and Dr. John G. McAfee, regarding the radiological diagnosis of adrenal tumors.

A major portion of the text is concerned with the diagnosis and management of conditions within the spectrum of gonadal dysgenesis, from overt, morphologic intersexuality to gonadal aplasia and its variants. Female hermaphroditism, whether due to congenital adrenal hyperplasia or that which is not associated with disordered adrenal metabolism, is likewise dealt with extensively and much of the available information regarding metabolic defects in the former condition is included.

There is a rather comprehensive review of the surgical aspects of hermaphroditism, wherein techniques employed in the reconstruction of the external genitalia are described in some detail. Similarly, a chapter is devoted to an excellent discussion of cryptorchidism and its management, stressing the high incidence of spontaneous testicular descent. The section dealing with endocrine tumors is well-organized, and while material concerning the surgical approach to adrenal disorders is rather brief, it is reasonably complete and includes descriptions and illustrations of several standard surgical techniques.

In short, this book is a very highly recommended reference.

ROBERT S. STEMPFEL, JR.
GERALD H. HOLMAN

Annual Review of Medicine, Vol. 9, 1958. Edited by DAVID A. RYTAND. 530 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

This volume is number 9 in a familiar series and departs little from its predecessors. Included among its contents are several authoritative reviews dealing with various infectious diseases and disorders of the skin, bones and joints, kidney, gastrointestinal tract, endocrine glands and the cardiovascular and nervous systems. Particularly interesting was Dixon's consideration of autoimmunity in disease. He presented a great deal of material of current clinical and laboratory interest and leavened it with his own critical comment. Currently pertinent is the chapter of Lasagna and Meier dealing with the clinical evaluation of drugs. The account of the recent advances of Soviet medical research represents a significant addition to the Annual Review of Medicine and hopefully represents a continuing effort to effectively disseminate knowledge of Soviet medical progress.

Certain of the papers, particularly the one dealing with paper chromatography and electrophoresis, presented bibliographic material almost exclusively. Such a chapter might well provide an interested reader with an extensive list of references only to deprive him of the attractive introduction to and fuller appreciation of an unfamiliar area of knowledge which the critical comments of a seasoned investigator would furnish.

ALBERT H. OWENS, JR.

Skin Grafting, 3rd edition. By JAMES BARRETT BROWN AND FRANK MCDOWELL. 411 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

This book, illustrating all of the major techniques of modern skin grafting in plastic surgery, is an outstanding contribution by the authors. Their experience in this field is well known throughout the world and this book represents a summation of their observations of great value, both to the general surgeon and to the specialist in the field of plastic surgery.

Several points of interest should be noted about this book. To the reviewer, it was immediately apparent that a strong and personal doctor-patient relationship existed in the care of the individuals illustrated in this book. The importance of this relationship in dealing with a major burn is well known to the surgeon, but has rarely received the emphasis given by Drs. Brown and McDowell.

There are many helpful additions to the second edition of their book and some of these should be noted. The principle of utilizing permanent pedicle blood carrying flaps to areas of low vitality, such as radiation defects or gun shot wounds is nicely illustrated. New techniques for skin grafting of the genitalia and interesting information on the development of techniques for the establishment of a skin bank in a modern hospital are included. The authors point out the occasional usefulness of early full thickness grafts to burned areas, replacing in some instances, the time consuming methods of debridement, split thickness grafting, physiotherapy and later full thickness grafting for final function. All of the treatment is consistently directed toward obtaining the maximum result from the standpoint of the "life time outlook for the patient".

Dr. Brown brings out an interesting point of view in suggesting that major burns should not be cared for in the modern hospital on a special burn ward. The chronic nature of these problems and the need for such patients to be surrounded by patients where average hospital stay is short was stressed by Dr. Brown. It is the feeling of the authors that this dispersal of burn patients may help impart "a certain sense of urgency" that would overcome the tendency for personnel to treat the hospital as a "second home for an indefinite period".

A fine section is included, pointing out the differences between civil and war injuries and this should be read by anyone concerned with problems of metropolitan civil defense planning. As, in their previous edition, the authors succeed in reducing surgical considerations to

simple and sound basic principles. There are three-hundred-twenty-eight excellent figures, including six color plates that illustrate beautifully the surgical procedures employed. The book is heartily recommended to anyone in the field of general and plastic surgery.

MILTON T. EDGERTON

Psychoprophylactic Preparation for Painless Childbirth. By ISIDORE BONSTEIN. 143 pp., $2.50. Grune & Stratton, New York, N. Y.

This is "an adaptation and sometimes a translation of the few French publications" on the subject by Lamaze and his collaborators. Based on Pavlov's work on the conditioned reflex, it is an attempt to apply this phenomenon to childbirth. Though strongly and correctly disclaiming any similarity to the "hypnotic" approach, the author also denies any resemblance to Grantly Dick-Read's method for decreasing the discomforts of childbirth though here the points in common seem far greater than the differences.

The book is particularly for the teacher of the method but reasonably satisfactory for use by the patient herself. "One man's meat is another man's poison". This reviewer finds much meat but feels sure the vast majority of obstetricians will reject it. However, its reported ten per cent failures (2 per cent in the hands of Lamaze) compares favorably with the failure rates of other methods and would seem to warrant serious consideration by all obstetricians.

EDMUND P. H. HARRISON, JR.

The Essence of Surgery. By C. STUART WELCH AND SAMUEL R. POWERS, JR. 320 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

This interesting little book is a curious mixture. Combined with broadly general philosophic discussions of surgical problems at a level suitable for the intelligent layman or first year medical student, there are detailed instructions in an admirable section on surgical technique for the use of hemostats, ligatures, directions for the sterilization of scalpels and discussion of the sizes of surgical sponges. There is an attempt to consider surgery as the branch of medicine which deals with injury and to describe the several types of injury which are arbitrarily given as loss of body tissue, loss of body fluids and infection, for the theme of the book is said to be that surgery is basically concerned with the problems of acute injury. There are brief sections of the book dealing with reconstructive surgery, and with surgery of malignant disease, neither of which would fit readily into the category of acute injury.

The attempt to write a broadly general book about surgery based almost entirely upon a discussion of principles is an exciting venture. It does not quite come off. In part because the authors have succumbed to the temptation of including conventional and unnecessary detail and in part because, conversely, the discussions are at times so general and broad as to lose much of their value. The section on fluid balance is particularly good.

There are no references except in the separate chapter on anesthesia. If the purpose of the book is to give the student a broad and general view of surgery early in his career it would seem it might be extremely useful to include a carefully selected list of references in each section.

MARK M. RAVITCH

Regulation and Mode of Action of Thyroid Hormones, Ciba Foundation, Colloquia on Endocrinology, Volume X. Edited by G. E. W. WOLSTENHOLME AND E. C. P. MILLAR. 311 pp., $8.50. Little, Brown and Co., Boston, Mass.

During recent years the Ciba Foundation for the Promotion of International Cooperation in Medical and Chemical Research has held forty conferences. The Foundation has published in book form the papers and discussions of thirty-four of these. This colloquium on the thy-

roid hormone is another of their excellent series. The material contained in this book will be of most value to those who are engaged in research on the thyroid gland and its hormones, but it will be also of interest to those who may wish to acquaint themselves with recently acquired knowledge and the newer postulates in thyroid physiology.

The first one-third of the book pertains to the relationship of the higher centers, such as the hypothalamus, on the pituitary-thyroid axis and a consideration of the pituitary's control of thyroid function. Harris of London and his associate, Woods, now again at Johns Hopkins, show that stimulation of the hypothalamus of normal rabbits results in increased thyroid activity and that this effect is accentuated in adrenalectomized animals. They suggest that their experiments, along with the results of the studies of others, indicate that if the adrenal cortex is not capable of responding to stressful stimuli, hypertrophy and hyperactivity of the thyroid may occur. Greer of California discusses the likelihood of the existence of two types of thyrotropic hormones, one producing thyroid growth dependent on an intact hypothalamus and the other stimulating thyroid hormone synthesis and release acting independently of the hypothalamus; but he concludes that there is insufficient information at present to establish such an hypothesis. In the examination of the cells of the anterior pituitary Purves and Griesbach of New Zealand have shown that thyrotropin exists in the basophils. Taurog, Long and Chaikoff of California have defined specifically the defects in thyroid hormone synthesis which occur in rats following hypophysectomy; and Halmi of Iowa delineates the factors which influence the ability of the thyroid to concentrate iodide. Such studies have added much to our knowledge of Graves' disease and may lead eventually to an understanding of its etiology.

The second portion of the book pertains to the various thyroid hormones and their metabolism. There are chapters on the enzymatic activity of the iodinated thyronines by Lissitzky and Bouchilloux of Marseilles, on the formation by rat kidney of the tetra- and triiodinated thyroacetic acids from thyroxine and triiodothyronine respectively by Lardy and his associates of Madison, and on the chemical substances produced by kidney and muscle in metabolizing triiodothyronine by Roche and his associates of Paris.

The final third section of the book relates to the metabolic effects of the thyroid hormones. Trotter of London has studied the properties of triiodothyroacetic acid in patients with myxedema. This compound, considered to be a major metabolic degradation product of triiodothyronine, accelerates the metabolism of myxedematous subjects; but it is less potent than triiodothyronine. Barker of Birmingham, Alabama, and Thibault of Paris have investigated the properties of the thyroid hormones on isolated tissues under various experimental conditions.

Although only a sketch of the content of this comprehensive treatise has been drawn, the student of the thyroid will be attracted to the fine print discussions following each presentation, for here are some of the most stimulating sections of the book. Finally, of considerable value are the index and the references; the latter not only follow each chapter but also are used to document statements throughout the discussions.

SAMUEL P. ASPER, JR.

BOOKS RECEIVED FOR REVIEW

Callander's Surgical Anatomy, 4th edition. By BARRY J. ANSON AND WALTER G. MADDOCK. 1157 pp., $21.00. W. B. Saunders Co., Philadelphia, Pa.

Chronic Illness in the United States, Vol. IV. Chronic Illness in a Large City. By Commission

on Chronic Illness. 620 pp., $8.00. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass.

A Classified Bibliography of Gerontology and Geriatrics. Supplement One 1949–1955. By NATHAN W. SHOCK. 525 pp., $15.00. Stanford University Press, Stanford, Cal.

Cold Injury, Ground Type. By COL. TOM F. WHAYNE AND MICHAEL E. DEBAKEY. Prepared under the direction of Maj. Gen. S. B. Hays. Editor in chief, Col. John Boyd Coates, Jr., Associate Editor, Elizabeth M. McFetridge. Office of the Surgeon General, Dept. of the Army, Washington, D. C. 570 pp., $6.25. Superintendent of Documents, U. S. Government Printing Office, Washington, D. C.

Emergency Treatment and Management, 2nd edition. By THOMAS FLINT, JR. 539 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Eye Surgery, 3rd edition. By H. B. STALLARD. 899 pp., $18.00. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Handbook of Respiration. Analysis and Compilation by PHILIP L. ALTMAN, JOHN F. GIBSON, JR. AND CHARLES C. WANG. Edited by Dorothy S. Dittmer and Rudolph M. Grebe. Prepared under the direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, The National Academy of Sciences, The National Research Council. 403 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Hospital and Community. A. History of the Royal Melbourne Hospital. By K. S. INGLIS. 226 pp., $5.50. Cambridge University Press, New York, N. Y.

Infectious Diseases of Children. By SAUL KRUGMAN AND ROBERT WARD. 340 pp., $10.00. The C. V. Mosby Co., St. Louis, Mo.

An Introduction to Physics in Nursing, 3rd edition. By HESSEL H. FLITTER. 253 pp., $3.75. The C. V. Mosby Co., St. Louis, Mo.

Introduction to Psychiatric Nursing, 2nd edition. By MARION E. KALKMAN. 331 pp., $5.95 McGraw-Hill Book Co., New York, N. Y.

The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine. A Chronicle. Vol. II, 1893–1905. By ALAN M. CHESNEY. 499 pp., $6.00. The Johns Hopkins Press, Baltimore, Md.

Mosby's Comprehensive Review of Nursing, 4th edition. 788 pp., $7.50. The C. V. Mosby Co., St. Louis, Mo.

Physical Examination of the Surgical Patient, 2nd edition. By J. ENGLEBERT DUNPHY AND THOMAS W. BOTSFORD. 375 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

The Principles and Practice of Medicine, 4th edition. A Textbook for Students and Doctors. By SIR STANLEY DAVIDSON and the Staff of the Department of Medicine, University of Edinburgh, and Associated Clinical Units. 1067 pp., $8.00. The Williams & Wilkins Co., Baltimore Md., exclusive U. S. agents.

Dr. Squibb, the life and times of a rugged idealist. By LAWRENCE G. BLOCHMAN. 372 pp., $5.00. Simon and Schuster, New York, N. Y.

Technic and Practice of Psychoanalysis. By LEON J. SAUL. 244 pp., $8.00. J. B. Lippincott Co., Philadelphia, Pa.

Therapeutic Exercise. Edited by SIDNEY LICHT. Volume III of Physical Medicine Library. 893 pp., $16.00. Elizabeth Licht, Publisher, New Haven, Conn.

To Work in the Vineyard of Surgery. The Reminiscences of J. Collins Warren, 1842–1927. Edited, with appendices, notes and comments by EDWARD D. CHURCHILL. 288 pp., $6.00. Harvard University Press, Cambridge, Mass.

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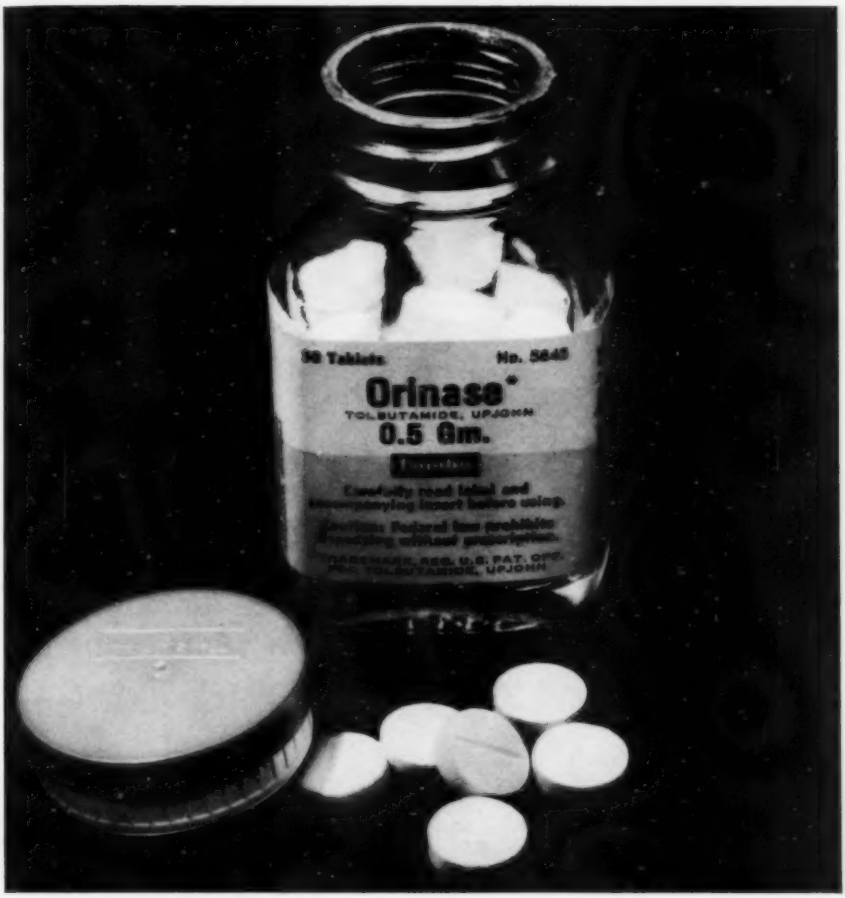

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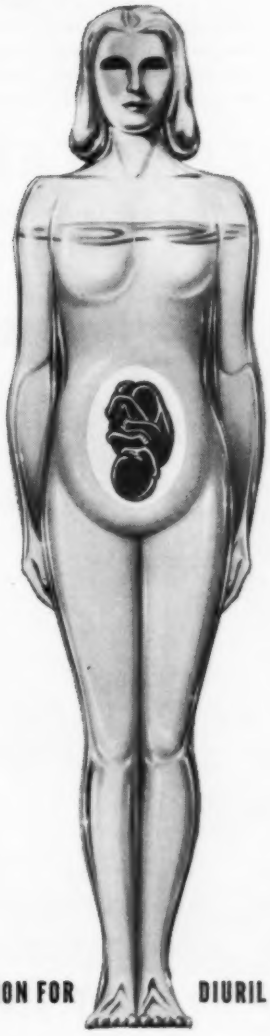

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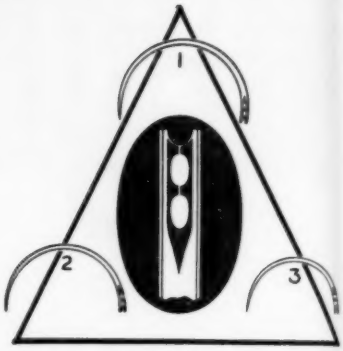

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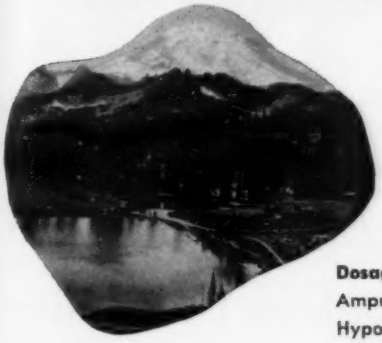

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